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## CLINICAL AND EXPERIMENTAL

### THE RELATION OF THE CLINICAL PATHOLOGIST TO THE HOSPITAL*


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WITHIN the last five years a revolutionary change has been effected in the hospitals of this country. Formerly serving as mere boarding houses for the sick, they now endeavor to supervise and guide the medical treatment of patients entrusted to their care. The movement for the betterment of hospitals has been sponsored by both medical and lay organizations interested in the welfare of the patient and in the advancement of scientific medicine. Thus the combined efforts of the American College of Surgeons, the Catholic Hospital Association and the American Hospital Association have brought about a remarkable transformation in the conduct and management of institutions for the care of the sick, eliminating abuses that had crept in and stimulating the medical staff to better efforts.

The program laid down for a so-called standardized hospital embraces three main features: (1) A properly organized medical staff meeting at regular intervals for the discussion of problems or cases in the hospital; (2) maintenance of a good system of case records, and (3), facilities for x-ray and clinical laboratory examinations.

It is with the very last item that we are here concerned, namely, the place of the laboratory, and especially of its director in the hospital standardization scheme. As the movement for the betterment of hospitals is still young, virtually in the making, many minor and even essential details in the program are still to be worked out. What routine examinations are to be made on every patient, medical or surgical, other than those especially requested by the clinician, what tissue removed at operation is to be sanctioned, and similar questions still await authoritative definition. The chief

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problem, however, resides in the famine of competent pathologists. The standardization movement making laboratory examinations obligatory disclosed, in the surveys made, a woeful lack of physicians who were specializing in this branch of medicine and who were eligible for the vacant positions. It may be safely asserted that one-half of the hospitals in the United States are functioning without the services of a laboratory director. To supply the deficiency and comply with the letter of the rule, hospital authorities and medical staffs have resorted to the doubtful expediency of substituting untrained internes or lay technicians. It is unfortunate that in the domain of clinical pathology more stress has been laid on the laboratory, i.e., the physical equipment, than on the mental endowment and ability of the director. It is, as if the success of a surgical operation were measured by the glitter of the beautiful operating room rather than by the judgment, knowledge and skill of the surgeon. The medical profession has been improperly educated to view the work of the clinical pathologist as something impersonal—an entity passing through a machine unknown at its entrance, and labeled with a diagnosis at its exit. With this kind of an outlook, what was more natural than to have a technician of more or less training to fill in the gap? The great dearth of clinical pathologists may be ascribed chiefly to the economic factor which often determines the physician's specialty as it does, in general, man's vocation in life. The reward held out to the clinical pathologist is too meager to entice the recent graduate or the practitioner from the more lucrative branches of surgery or internal medicine. His status from the standpoint of finance, as well as of dignity, has not been such as to lure him into this field. The years of study required to familiarize himself with the sciences of chemistry, bacteriology, immunology, serology, clinical microscopy and, most difficult of all, tissue pathology, chill the enthusiasm of the medical student who may contemplate taking this branch up as his life's work. He will hesitate when he sees clinical pathology regarded as a handmaiden to medicine, and the pathologist in the rôle of a technician when in truth he is a consultant, and his specialty the most exacting of the branches of the healing art. Not a little to establish this distorted view of our calling has been contributed by the advertising of commercial laboratories in our medical press, who have cheapened and degraded clinical pathology by their blatant self-praise and crying out their fees in bargain counter style. If the standardization of hospitals is to endure, if scientific medicine is to take the place of empiricism, if exact methods in the diagnosis of disease are to replace the symptomatic and expectant regime of old, then the rôle of the clinical pathologist in the hospital must be placed on a secure footing. The laboratory, figuratively speaking, is the keystone in the arch in the hospital structure. The interpretation and diagnosis by the pathologist determines the weal and woe of the patient. An enormous responsibility rests on his shoulders which cannot be relegated to incompetent hands. A wrong diagnosis in tissue pathology may spell a tragedy, and while the clinician is an efficient check,

he frequently leans on the opinion of the pathologist in carrying out important operative or medical procedures.

What are the functions of the clinical pathologist in the hospital scheme? His duties are not ended when he or his technicians carry out the laboratory tests ordered by the clinician. Once more it should be emphasized that the pathologist is a consultant in medicine and as such the laboratory should be a meeting place for the attending physician to talk over cases with him. Both are equally interested in the welfare of the patient and his happy recovery. The pathologist not being in competitive practice and devoting his energies exclusively to his branch, usually enjoys the confidence of the hospital authorities and the clinician. He is therefore in a position where he is cordially welcomed by the attending physician to see the patient with him, discuss the symptoms and may therefore tactfully suggest the necessary lines of laboratory investigation which may throw further light on an obscure case. Experience has shown that the clinician is grateful for such help. The post held by the laboratory director is such as to enable him to stimulate scientific work in the institution if he but take the initiative. He should interest himself actively in the scientific program of the staff meetings, when he may speak of new laboratory procedures or recent original research in this field, or present specimens of pathologic tissue removed at operation or autopsy. He should encourage more frequent postmortem examinations, by reminding the attending physician at the proper time.

The pathologist is called on more frequently than any member of the staff to help the standardization program in the matter of records. By serving on the chart committee and by his readiness to give counsel to the record clerk, he furthers the cause of scientific medicine. The statistical data of the laboratory should be carefully kept and compiled. The information furnished, especially those relating to tissue pathology, when collated with similar data from other standardized hospitals, will form a valuable mine for study and research.

Lastly, the pathologist should be a teacher. He should encourage the resident internes to follow clinical findings with the laboratory investigations. In addition to assigning each interne a service in the laboratory during his period of training, seminars for the house staff should be held at frequent intervals to discuss laboratory findings and discuss pathologic specimens.

In pointing out in this fragmentary manner the activities of the hospital pathologist, I have had in mind not the large teaching institutions affiliated with medical schools, but the average private hospital of fifty beds and over, such as are found in most of the larger communities.

A difficult question arises when we come to the subject of the compensation of the laboratory director for his services. It may seem in poor taste to discuss financial matters in connection with such an elevated theme as clinical pathology, but in this work-a-day world ideals are inseparably bound up with the economic factor. It is quite evident that the clinical pathologist in order to do effective work should not be distracted by the exactions of

private practice, but should devote himself exclusively to his specialty. The compensation for his hospital connection should be such as to free him from the temptation to enter the fields of surgery or internal medicine for which his knowledge and training in clinical pathology form a splendid background. This leads to the next question: What should be the source of the pathologist's pay, the hospital or the patient? Various methods are pursued in different institutions both in the way of raising the necessary funds for conducting the laboratory and also in the manner of compensating the pathologist. As the standardization movement found the hospitals unprepared for the new requirements, there was no precedent to go by, and each institution is endeavoring to solve the problem in its own way.

As to raising the funds, most institutions charge a flat fee to the patient, either based on the price of the room, or the same fee to all except ward patients, which are usually charged half of the private room rates. While some hospitals furnish all kinds of laboratory service for this flat fee, others charge an additional fee for other than routine urine and blood examinations, approximating more or less to the prevailing fees of outside private laboratories. A few hospitals make no charge at all for laboratory service, covering the expense from the general fund or special endowment.

Regarding the compensation of the pathologist, opinions differ as to whether a salary or a share of the laboratory income is the best procedure. Both systems are in vogue. The argument against salary is based on the general aversion of the medical profession to have their calling socialized. If the process be started with the clinical pathologist, there is nothing in the way of continuing the experiment with the surgeon and other specialists. The clinician with the best of intentions desires the laboratory service to be given at a minimum cost so as not to throw an added burden on the patient who finds hospital accomodations expensive This applies, of course, to the middle class who form the great majority of his clientele. The hospital, too, usually is averse to raising the fees to meet the added laboratory cost. Economic conditions thus militate against a fair and adequate compensation for the pathologist, and the salary system will tend to lower it and drive the best men from the field.

The patient will have to pass through a period of education to the necessity of laboratory aids in diagnosis, and corresonding reward for the pathologist, the same as for any other consultant. In my humble opinion, his services should not be carried as overhead expense, such as light and heat, but the patient should receive on his bill a separate item for laboratory consultations. A moderate flat rate may be charged for the usual obligatory routine examinations of the urine and blood, but operations requiring skill and interpretation based on knowledge and experience should be appraised at their proper value. After due allowance is made for the current expense of conducting the laboratory, the pathologist should receive a considerable share of the laboratory income with due regard to the pro rata of the hospital in capital investment, technicians' salaries and other incidentals. This

method will preserve the dignity of the pathologist, maintain his individualism and stimulate him to the best efforts.

The physicians of a community that boasts of a hospital of 100 beds should see to it that the clinical pathologist connected with the institution be encouraged by adequate compensation to continue and perfect himself in his specialty. Only by proper support of the laboratory director will the hospital conform to the standardization program and fulfill his highest mission of the greatest good to the patient.

*(For discussion, see p. 423.)*

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## THE PATHOLOGIST OF THE TWENTIETH CENTURY*

By William Carpenter MacCarty, M.D., Rochester, Minn.

MUCH confusion of ideas about the status of the individual whom we have called the pathologist exists in the medical profession; and it is of such a character that actual inefficiency exists in the rendition of service to those who suffer from disease. This fact, which I have learned from practical experience, stimulates me to attempt to show the real status of the pathologist in our professional activity. This problem involves a study of the history of the evolution of our profession, the evolution of the various specialties, a study of fundamental principles of education, especially medical education, and a consideration of the practical, educational, and investigative features of all work which necessitates laboratories.

The vocation which we call medicine is a mixture of art, some science, philosophy, religion, and commercialism (in its broad sense). It is not an art, a science, a philosophy, a religion, or a business. It involves research, teaching and the practical application of facts to the individual suffering from disease. As a vocation it is of increasing economic value to our social organization. As a specialty, medicine has evolved out of the functions of the ancient priesthood. Its growth has been slow; its greatest progress has been dependent upon periodic increases in our knowledge of anatomy, physiology, and morbid anatomy, and the application of this information to the recognition, treatment, and prevention of disease. There has been no period when our knowledge may be spoken of as fixed; it is, therefore, not to be classed with mathematics and physics which perhaps represent our most fixed knowledge. It is, as a branch of knowledge, a mass of things—some of which are true, mixed with things which may not be true; it is clothed in a language not universally intelligible even to those who are designated Doctors of Medicine; it is unintelligible to biologists despite the fact that it is a biologic science. It has rapidly grown from a specialty, the functions of which were carried out by a single individual to one, the functions of which

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involve knowledge of chemistry, physics, bacteriology, botany, entomology, embryology, comparative anatomy, serology, immunology, protozoology, physiology, normal gross anatomy, morbid gross anatomy, histology, histomorbid anatomy, cytoanatomy, cytomorbid anatomy, sanitation, electricity, surgery, philosophy, art, and business.

In the development of these specialties, there was a period when an individual—who had the training of a physician and surgeon, and had also added to this a special training in morbid anatomy—was called a pathologist simply because he knew the most about disease. Thus in the middle of the nineteenth century the pathologist happened to be a morbid anatomist. At the beginning of the twentieth century how many of those who are called pathologists are expert morbid anatomists? Do these so-called pathologists know the most about disease? They are today bacteriologists, immunologists, serologists, parasitologists, laboratory technicians, and teachers. Most of the occupants of the Chairs of Pathology teach some morbid anatomy from autopsy material but they do their investigative work in bacteriology, serology, immunology or public health. Rarely, if ever, do they study human morbid anatomy during the life of the patient. Their knowledge of morbid anatomy is based on the end results of disease and not upon disease in the process of affecting the individual while alive.

Our activities in the medical profession are so specialized that soon we will be just as ridiculously specialized as the rest of human labor. With this specialization has come increased cost of production of physicians and service, narrowness of knowledge, and a certain selfish indifference to the immediate general care of those who suffer from the commoner and less serious diseases.

Necessity for efficiency, applied to medicine, allows and demands that we occasionally take stock of the existing states of teaching, research and practice. Such an analysis demands, also, that we divide our knowledge and activities into certain groups: (1) Those of actual value in treatment and prevention of disease. (2) Those of value for teaching. (3) Those for research.

These fundamentals occur to me when I try to determine what a pathologist has been and what he is today. The activities of most pathologists fall into the last two groups; they have left the first to physicians and surgeons who may or may not (usually the latter) have spent a short time studying dead house morbid anatomy which—although it reveals many interesting, and valuable, facts—never reveals facts of value to the individual while suffering from the disease.

The question arises: Is there no place for an extensive knowledge of morbid anatomy during the life of the patient? The answer, of course, is yes, because our primary object is the patient. If this be true why not utilize those members of our profession with the greatest knowledge of living morbid anatomy in the actual dealing with living patients? Why allow those of our profession who know only a small amount of living or dead mor-

bid anatomy, to decide upon the practical diagnosis and treatment of disease? Is it more difficult, and does it take a longer time to become expert in history writing, external inspection, auscultation, and palpation than it does to become expert in the recognition and application of the facts of morbid anatomy and physiology? The fact that in a series of 16,000 patients 24.5 per cent of the clinical preoperative diagnoses or summaries were put down as questionable, and also the fact that these same clinicians required 225,785 laboratory tests on 60,645 patients show that clinicians recognize that the diagnosis of disease is no longer covered by history, inspection, palpation, and auscultation.

I think now of another question: How many clinicians check their diagnoses and find out their real efficiency? Just a few figures might be of value in showing the actual clinical diagnostic efficiency in some rather common pathologic conditions.

In 1000 preoperative clinical summaries (or diagnoses) there were 130 (13 per cent) which were pathologically incorrect, although in none of these did the incorrectness affect the patient's welfare for two large reasons: the surgeon had an opportunity to explore—a method of increasing clinical vision. The surgeon has learned, by experience, to recognize morbid conditions better than pure clinicians because he has a greater opportunity for wider vision during the life of his patient. In many respects he is a better pathologist (in its broad sense) because his knowledge of disease, as it really is in the premortem stage, is much better than that of the pure clinician who rarely visits the operating room and too infrequently pays any attention to morbid anatomy, even in the autopsy room. The second reason why these 130 patients which had been diagnosed incorrectly did not suffer ill effects, was the consultation, at operation, with a clinical morbid anatomist who is primarily interested in immediate service during the life of the patient rather than after the patient has died. This individual had learned from experience in the autopsy room and the operating room that in the former, one does not see the early manifestations of disease, which one sees at operative explorations. Under proper circumstances of clinical laboratory organization such a morbid anatomist can avail himself, not only of gross material, but also of microscopic material, and indeed, a study of living tissues which are quite different from those upon which our present teaching of pathologic anatomy is based. I might give you many figures to show the percentage of clinical diagnostic error but I wish merely to draw your attention to the fact that clinicians are beginning to recognize their inability to make a certain large percentage of diagnosis by signs, symptoms, blood counts, and urine tests alone. Even with all of the modern so-called clinical and clinical laboratory methods for diagnosis those physicians who are conscientious make questionable diagnoses in a very high percentage of cases. Thus in benign conditions of the stomach this percentage is 16, in malignant conditions of the same organ it is 13.8 per cent; in benign uterine conditions it is 35.6 per cent and in malignant conditions in this organ it is 77 per cent.

It is quite true these figures represent those after he has discarded (correctly or incorrectly) the inoperable cases and those in which signs and symptoms were absent or not sufficient to make him suspect a pathologic condition. The suggestion might be ventured that I believe—and in some of those cases I know—that the proper diagnosis might have been made by one well versed in living morbid anatomy if that morbid anatomist had trained himself in clinical manifestations of the subject of his expertness.

This brings us to the main point: namely, the pathologist of the twentieth century who, I think, should be still the one who knows the most about disease—the one who has studied disease from the greatest number of angles—the one who has spent a long apprenticeship in the various fields involved in diagnosis, treatment, and prevention. To me Bright, Hodgkin, Laennec, Louis, Bichat, Addison, Fagg, Osler, Paget, Billroth, Gross, Fenger, Halsted and others were great pathologists: they knew all that was known about some diseases from first hand experience. They did not know signs and symptoms alone, or the morbid anatomy alone, or etiology alone, or surgical technic alone. Their knowledge was not taken from books.

The tendency of the times is away from such training. For awhile professors of medicine were chosen from pure clinicians, later morbid anatomists, and now from serologists, physiologists or physiologic chemists. Only too frequently these modern heads of medical university departments are young men who have not had great contact with patients; most of them have made their reputations upon intricate limited fundamental chemical, physiologic, or bacteriologic problems, to which they have devoted most of their time since graduation. Valuable as these have been—and they most certainly should be encouraged—they frequently occupy much time which should be utilized in sifting out the practical things of immediate value, and systematizing these for teaching of young men to care for the patients who are now drifting to osteopaths, chiropractors, and Christian Scientists. I might say here that I have no objections to osteopaths, chiropractors, and Christian Scientists. Many of them are conscientious and do some good. I do not believe in fighting competitors by slander or legislation. The best service, rendered in the most economic manner, should be our motto in the medical profession.

Experience in teaching and examining many hundreds of graduates of Class A schools has taught me that much valuable young brain power of students is wasted by many teachers who have held the ideal of research paramount. The pathologists I have mentioned were great clinicians; they inspired research and did research but they kept contact with the general common needs of the public and did not fail to make their students practical doctors. The pathologist of the twentieth century must know clinical signs and symptoms, morbid anatomy; bacteriological, serological, immunological, chemical, physical, economic, social, and psychological facts directly connected with the immediate practical problems of disease if he is to be an influential teacher, investigator, or practitioner of medicine. Extensive experience with the hydrogen-ion concentration of the blood, experimental pro-

duction of Eckt fistulae, the determination of the bacteriologic etiologic factors in "flu," the microscopic contraction of vascular capillaries of an organ or the discovery of renal, hepatic, or splenic functions by some blood test will not make a great pathologist and may be of little value in teaching students who expect to treat the many common diseases. Let us not forget the big problems in our enthusiasm for very limited specific progress. Let us always divide our knowledge into that which is of immediate value to treatment and prevention of disease, that of value in teaching and that of value in investigation. The real pathologist will have his knowledge thus classified; and only upon such a classification can he become the great teacher and inspiration in our field of medicine.

The greatest pathologist in the twentieth century will be the greatest authority upon disease; and the greatest authority will be the greatest authority, whether he be secondarily adept in laboratory methods, teaching, investigation or practice.

*(For discussion, see p. 425.)*

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## DIAGNOSTIC METHODS IN NEUROSYPHILIS*

By Leon H. Cornwall, M.D., New York, N. Y.

THE time was, not so long ago, when the diagnosis of syphilis of the nervous system was made in three ways: first by the occurrence of a clear cut syndrome that was so generally recognized to be associated with antecedent syphilitic infection as to justify a *post hoc propter hoc* deduction; second by the sudden onset of impaired neurologic function during the course of active syphilitic infection and the third by elimination.

Among the syndromes which have been historically associated with syphilitic infection, tabes dorsalis and general paresis are the most familiar. They were formerly considered as meta or parasyphilitic diseases but their recognition as syphilis *per se* was strongly suspected after the classical studies on the spinal fluid by Quincke and the amplification by Fournier, Nonne, Rehm, Plaut, Schotmüller, and others. The adaptation by Wassermann of the complement-fixation studies of Bordet-Gengou followed by the application of the Wassermann test to the spinal fluid strengthened the conviction of the etiology of these syndromes and the confirmation came with the discovery of the Spirocheta pallidum in the nervous tissue by Noguchi and Moore.

Subsequent studies by European, English and American observers have rationalized our conception of neurosyphilis, and clinical pathology has furnished methods to enhance clinical observation. These methods are only aids in diagnosis. They must be used as adjuncts to clinical observation but never as substitutes. When so employed they strengthen diagnosis and conduce to

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greater accuracy in prognosis. When not so employed they encourage intellectual lassitude and diagnostic error.

In cases of impaired neurologic function originating during the course of recognized syphilitic infection a combination of clinical observaton with pathologic studies of the blood and spinal fluid broadens our concepts of the pathologic type and anatomic location of the lesions with which we are dealing. A repetition of these correlated studies after the institution of therapy gives us information concerning the therapeutic response which warrants prognostic speculation.

Long before the laboratory furnished the methods of precision that we now possess, clinical observers taught that certain symptoms in the absence of trauma, neoplasm, toxemia, acute infection, anemia or local causes were indicative of syphilis. Among them might be mentioned pupillary inequalities, impairment or loss of the light reflex with preservation of accommodation, miosis, cranial nerve pareses and palsies, especially of the oculomotorius and abducens, appendicular pareses and palsies in youth and early adult life, epileptiform seizures beginning in the third or fourth decade, and so on.

Our methods of today have added merely a link to the chain. Positive laboratory findings alone do not always justify a final diagnosis until other possibilities have been eliminated. Negative laboratory findings alone do not justify the elimination of syphilis as a possibility until many other considerations have been weighed, among which might be mentioned physical findings, family history, antecedent personal history, and antecedent therapy. With all of these facts at hand the interpretation rests upon the correlation of clinical and laboratory findings.

At this point we should recall that the nervous system proper is derived from the embryonal ectoderm. These ectodermal derivatives consist of neural parenchyma and neural supportive structures. By parenchyma we mean the neurones which in turn consist of nerve cells, axis cylinders, dendrites, and myelin sheaths. The supportive structures are the glia cells and glia fibers.

The meningeal coverings of the nervous system and the vascular elements are mesodermal derivatives.

In accordance with the derivation of the structures involved we may classify neurosyphilis then as mesodermal, ectodermal or mixed. To indicate structural location more specifically we may employ terminology indicative of this: meningeal, vascular, meningovascular, parenchymatous.

To indicate topographical location we may specify peripheral or central and subdivide the latter into cerebral, spinal or cerebrospinal.

Having illustrated the nomenclature that will be employed in this discussion let us consider next the diagnostic methods applicable to neurosyphilis and attempt to evaluate them.

It is scarcely necessary to mention that positive blood serology alone merely suggests the possibility of the subsequent development of neurosyphilis. Positive blood and negative fluid serology together with clinical signs

associated with neurosyphilis requires the elimination of other etiologic factors. Positive blood and fluid serology always mean neurosyphilis.

Negative blood serology should never lead to the exclusion of a diagnosis of neurosyphilis in the presence of suggestive clinical signs. Negative blood and positive fluid serology may occur in 5 per cent to 10 per cent of cases depending upon the duration of the infection and the amount of therapy that has been administered. This combination is not infrequently encountered in mesodermal neurosyphilis that manifests itself in the secondary stage during the course of the therapy.

The following case is an illustration:

E. R., age twenty-six, primary and secondary manifestations in January, 1920. From January to December, 1920, treatment consisted of 17 intravenous arsphenamine injections. The blood serology was rendered negative.

On February 28, 1921, this case was referred to the Neurosphilis Clinic because of excruciating headache that persisted day and night producing insomnia and well defined anxiety features. The neurologic examination revealed general hyperreflexia and dilated pupils as the only objective symptoms.

The spinal fluid examination revealed:

normal color
2-plus pressure
541 cells
3-plus globulin
4-plus Wassermann with 0.5 c.c.
Colloidal gold 3334455432

This exemplifies a case of cerebral mesodermal neurosyphilis originating early in the disease. The lesions were of the acute exudative variety and judging from the accompanying clinical features, were producing their principal effects over the convexity of the hemispheres. It might be remarked that under combined intravenous and intraspinous therapy the cells dropped to 17 and the Wassermann in the fluid became negative within eight weeks. The headache was improved after one lumbar puncture and disappeared entirely after the second.

Negative findings in the blood and spinal fluid in the absence of a clinical history of syphilitic infection require strong clinical confirmation for the diagnosis of neurosyphilis. The following case is in pont:

M. L.—Age forty-seven—Negative syphilitic history.

Symptoms: Right hemiparesis. Completo personality change. Slovenly personal habits. Speech slow and suggestive of the paretic type. Memory and association defects. Emotional in stability.

Neurologic examination negative except for tremor of facial muscles and the hemiparesis.

The blood was negative on eight successive examinations before and after provocative therapy. The spinal fluid was negative except for a trace of globulin and 17 cells.

After several months of observation the diagnosis of cerebral arteriosclerosis was made.

The absence of physical signs of organic defect of the nervous system in a case with positive blood serology does not rule out neurosyphilis. Psychoneurotic manifestations on a luetic basis are common.

A. D.—Age twenty-six—Husband a known syphilitic. History of one miscarriage at 5 months. The complaints were pains in shoulders and arms and fear of paralysis or insanity. There were no objective signs of neurologic defects. The blood was 4-plus and the spinal fluid revealed increased pressure, 10 cells, normal globulin, Wassermann 4-plus with 1.5 c.c. and colloidal gold 1233322111.

This was a case of anatomically asymptomatic mesodermal neurosyphilis with an anxiety neurosis.

Vascular syphilis is frequently unaccompanied by positive findings in the spinal fluid. The blood may be positive or negative depending upon the therapy. The neurologic symptoms are dependent upon the location and extent of the vascular lesions. In general, syphilis of the larger cerebral vessels produces neurologic symptoms but when confined to the smaller vessels psychiatric symptoms are the rule.

In short, vascular neurosyphilis is merely one part of generalized vascular syphilis reflecting a symptomatology dependent upon the anatomic location.

Ectodermal neurosyphilis, of which general paresis, taboparesis and tabes dorsalis are the most familiar examples, is that type in which the pathology is degenerative and involves the neural components of ectodermal derivation, viz.: the neurones and neuroglia. Mesodermal neurosyphilis of long standing may compromise ectodermal structures but it is in the nature of a secondary process. For instance in cases of syphilitic meningitis similar to the one noted above, areas of exudation may extend from the pia into the cortical substance or if the meningitis involves the spinal cord exudative areas may extend into the cord substance. Without proper therapy these areas become transformed into scars. When this stage is reached the damage is permanent.

In ectodermal neurosyphilis, on the other hand, the ganglion cells suffer the brunt of the damage and conduction pathways are impaired as a result of the functional inactivity of either the sending, receiving or relaying stations.

Let us attempt briefly to evaluate the significance of the several laboratory procedures that the clinical pathologist has at his disposal.

The physical appearance of the spinal fluid in syphilis is usually clear, although it may be slightly opalescent due to increase in cellular or solid constituents. Xanthochromia may rarely be found in case of cord compression from gumma or loculation.

The pressure is nearly always increased in the early stage of invasion of the neural structures and especially in meningeal syphilis. Cerebral parenchymatous syphilis with the paretic syndrome is usually accompanied by high pressure.

In active meningovascular syphilis of any part of the neurospinal axis and in active spinal parenchymatous syphilis of the tabetic type the pressure is increased in a large percentage of cases. In latent or so-called "burned out" neurosyphilis the pressure is usually normal. In the vascular types the pressure is invariably normal.

The cell count is a clear indication of the acuity of meningeal involve-

ment. Pleocytosis is greatest in acute syphilitic lesions of the meninges and these lesions occur most frequently in the early stages of the disease. The predominance of lymphocytes, usually 90 per cent or more, does not differentiate syphilis from tuberculosis, poliomyelitis or encephalitis. The number of cells does, however, point to the location of the pathology.

Severe meningeal reaction (100 to 500 cells) speaks for superficiality of lesions and hopeful prognosis. A low cell count early in the disease with few or no clinical symptoms bespeaks a slight invasion and hopeful prognosis. A low cell count late in the disease with positive clinical findings and positive Wassermann, globulin and colloidal gold usually indicates deep-seated lesions with chronic meningeal changes.

Increased globulin in the spinal fluid, in and of itself, merely indicates altered physiology due to some irritant. Positive globulin alone with other fluid findings negative, but with clinical signs and positive blood serology, or negative blood serology in cases of proved syphilis, suggest vascular pathology.

A positive Wassermann reaction in the spinal fluid means neurosyphilis. If positive in amounts as small as 0.1 or 0.2 c.c. and not altered by therapy it usually indicates lesions of the ectoderm or mixed lesions of mesodermal and ectodermal elements. The persistence of a positive Wassermann in the smaller amounts for a period of one year or longer with globulin excess and low cell count despite active intravenous and intraspinous therapy speaks strongly for cerebral parenchymatous neurosyphilis. The paretic syndrome may develop at any time. In this connection it might be said that general paresis is a *clinical syndrome* due to syphilitic disease affecting the parenchymatous structures of the cerebral cortex.

The colloidal gold reaction is of extreme value but probably the most abused of all the tests that are applied to the spinal fluid. This reaction alone cannot diagnose neurosyphilis. The so-called paretic curve may occur in multiple sclerosis, lateral sclerosis, neoplasm, and septic meningitis. I have observed it once in a case of eclampsia.

The so-called luetic curve may occur in anterior poliomyelitis, epidemic encephalitis, multiple sclerosis, meningomyelitis and metabolic diseases.

The so-called meningitic curve may occur in any of the subacute meningeal lesions.

When the so-called paretic curve is present and there is a positive fluid Wassermann and psychotic manifestations it indicates ectodermal syphilis involving the cerebral cortex. The persistence of this same reaction under therapy with a positive Wassermann in the smaller amounts suggests a bad prognosis and a typical paretic psychosis may develop suddenly at any time.

There is no such thing as a tabetic curve with the colloidal gold. All types of reaction may be obtained in tabes dorsalis depending upon the duration and extent of the lesions and therapy.

Colloidal gold results must be interpreted in conjunction with the other laboratory and clinical findings. The changes in the reaction under therapy

is a valuable prognostic guide. One result has but slight value and without a cell count, globulin and Wassermann is practically valueless. Repeated results when linked with clinical findings and other laboratory findings are of extreme value in forming a broad conception of the type of pathology and its prognosis.

*(For discussion, see p. 425.)*

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## THE VALUE OF GERMANIUM DIOXIDE AS A THERAPEUTIC AGENT IN THE ANEMIAS OF CHILDHOOD*

By Herbert W. Schmitz,† M.D., New York

THE beneficial therapeutic effects of arsenic and its various preparations on the blood and blood-forming organs have been recognized for many years. The position of germanium adjacent to arsenic in the periodic system suggested to Hammett, Müller and Nowrey the possibility that this element might exhibit similar properties. Its existence, atomic weight, and chemical properties were foretold by Mendeléeff[1] in 1871 by the aid of the periodic law, but it was not discovered until 15 years later by Winkler[2] in the silver ore argyrodite at Freiberg, Germany.

Hammett, Müller, and Nowrey[3] in comparing the relative toxicity of germanium dioxide and arsenic trioxide for the mature albino rat found that the former can be given subcutaneously in doses up to 180 mg. per kilo of body weight with no apparent toxic effects, but that the latter generally proved fatal in doses of 8 mg. per kilo of body weight.

A study of the blood by the same investigators[4] following subcutaneous injections, revealed a marked and sustained rise in the number of erythrocytes; the increase ranging from 1 million to 5 million erythrocytes per c.mm. This increase occurred as a rule within a week's time and after one or two injections. A greater response was always noted in those test animals with low initial counts and vice versa, thus suggesting a relationship between the degree of response and the original number of red blood cells present. In comparing the degree of response with the size of the dose, no evidence was obtained to show that the larger doses exhibited a greater stimulating effect than did the smaller ones. Histologic examinations of the bone marrow[5] of the germanium-treated rats gave evidence of a marked increase of nucleated erythrocytes over the number present in the marrow sections of the controls. The circulating blood of the test animals likewise exhibited a greater number of young red cells than the blood of the control animals. No changes were noted in the white blood cells. Müller and Iszard[6] demonstrated a similar erythrocythemia following administration of germanium dioxide in the guinea pig, rabbit, dog, and man. In the case of man, the dose was administered orally, and apparently absorption occurred

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very promptly when given in this manner. Germanium dioxide does not accumulate in the system, but is readily eliminated through the kidneys and in small part through the alimentary tract. The lethal dose as calculated by Müller and Iszard[6] is about 586 mg. per kilo of body weight.

As Hammett and Nowrey[5] have pointed out, the cause of any given erythrocythemia may be either apparent or real; apparent, when the plasma volume is decreased, and real, when there is an increased production of new red blood cells, a division, or a sudden release of such cells from deposits in the various organs. Since in the germanium-treated rats the bone marrow showed an increased number of nucleated red cells, and the circulating blood of these same animals exhibited a greater percentage of young erythrocytes, their conclusion that germanium produces a real erythrocytosis in laboratory animals and normal man is justified. Further, this elevated red cell count was still present two weeks after the last dose was given, which fact speaks strongly in favor of a real erythrocytosis, for an apparent increase due to decrease in plasma volume would certainly not be as lasting in nature. Additional data in support of this contention are presented by Kast, Croll and Schmitz[7] who found that the increase of the total solids of the blood in cases of anemia treated with germanium generally ran parallel to the hemoglobin changes, but that the increase was not great enough to explain the erythrocytosis on the basis of a decreased plasma volume.

The therapeutic application of germanium was first undertaken by Kast, Croll and Schmitz[7] who reported observations on sixteen cases of anemia; four cases of anemia following hemorrhage, ten cases of secondary anemia and two cases of pernicious anemia. In some of the cases studied, especially in the cases of anemia following hemorrhage, a marked and sustained erythrocythemia was observed. The response of the hemoglobin to the germanium was usually more delayed and somewhat less marked than was the case of the erythrocytes. The white cell counts showed no significant changes. The Wassermann test and the coagulation time did not appear to be affected, and no changes in the functional activity of the kidneys was noted from examinations of the urine and blood before and after germanium therapy.

This paper presents the results obtained with germanium in thirteen unselected cases of anemia in childhood. The germanium was given by mouth in the form of the oxide in 0.2 per cent water solution. It was necessary to heat the solution for several minutes in order to dissolve the compound completely. No precipitation took place on cooling. The dose given ranged from 30 to 60 mg. daily, or every other day, until about 300 to 1000 mg. had been administered, based on 100 to 150 mg. per kilo of body weight. Erythrocyte counts and hemoglobin estimations were made preceding, during, and following the period of germanium treatment. Blood counting pipettes and hemocytometer with double Neubauer ruling, certified by the U. S. Bureau of Standards, were employed. For the hemoglobin estimations, the blood was treated with 0.1 N hydrochloric acid to form acid hematin, and after standing for several hours (Robscheit[8]) was compared with a standard in the Bock-Benedict colorimeter. The standard hemoglobin was prepared from blood whose oxygen carrying capacity was determined by Van Slyke's method.[9]

TABLE I

EFFECT OF GERMANIUM DIOXIDE ON ERYTHROCYTES AND HEMOGLOBIN IN ANEMIA

| CASE | DATE 1922 | GERMANIUM DIOXIDE MG. | BLOOD ERYTHROCYTES MILLION PER C. MM. | HEMOGLOBIN GM. PER 100 C.C.† | REMARKS |
|---|---|---|---|---|---|
| 1. A.K. | 7-5 | | 2.02 | 4.6 | Age 1½ years. |
| | 7-7 | 25 | | | Diagnosis—lobar pneumonia malnutrition, rickets, severe secondary anemia. |
| | 7-8 | 35 | 2.04 | | |
| | 7-9 | 50 | | | |
| | 7-10 | 50 | 2.32 | 5.05 | |
| | 7-11 | 50 | 2.59 | 5.55 | Temperature 100°F. at time $GeO_2$ was given; normal since 7/13/22. |
| | 7-13 | 50 | 2.76 | 6.20 | |
| | 7-14 | 50 | 2.62 | 6.0 | |
| | 7-15 | 50 | 2.81 | 6.1 | |
| | 7-16 | 50 | 2.75 | 6.1 | |
| | 7-17 | | | | |
| | 7-22 | | 3.18 | 6.7 | |
| | 7-24 | | 3.20 | 6.6 | |
| | 7-28 | | 4.02 | 7.5 | |
| | 7-31 | 25 | 3.55 | 7.2 | |
| | 8-1 | 50 | 3.80 | 7.2 | |
| | 8-4 | | 3.72 | 7.6 | |
| | 9-26 | | 4.96 | 12.0 | |
| 2. P.F. | 7-20 | | 3.53 | 6.5 | Age 2½ years. |
| | 7-21 | 30 | | | Diagnosis—empyema, pyelitis, secondary anemia. |
| | 7-22 | 30 | | | |
| | 7-24 | 50 | 3.01 | 6.6 | July 20, time of administration of $GeO_2$, pleural cavity still discharging moderate amount of pus. |
| | 7-26 | 50 | 3.36 | 6.35 | |
| | 7-27 | 50 | | | |
| | 7-28 | | 4.35 | 7.8 | |
| | 7-31 | 50 | 4.25 | 7.2 | Temperature 98° to 100°F. |
| | 8-1 | 50 | | | |
| | 8-2 | | 4.42 | 8.0 | |
| 3. D.R. | 11-21 | 30 | 4.85* | 7.0 | Age 1½ years. |
| | 11-22 | 30 | | | Diagnosis—pleurisy with effusion, secondary anemia. |
| | 11-23 | 40 | 5.10 | 7.2 | |
| | 11-24 | 40 | | | |
| | 11-25 | 40 | | | |
| | 11-27 | 35 | 5.13 | 7.0 | |
| | 11-28 | 40 | | | |
| | 11-29 | 50 | 5.35 | 7.6 | |
| | 11-30 | 50 | | | |
| | 12-1 | 45 | | | |
| | 12-2 | | 5.48 | 7.7 | |
| | 12-6 | | 5.20 | 7.9 | |
| 4. E.F. | 1-4-23 | 40 | 3.11* | 6.7 | Age 6 months. |
| | 1-5 | 40 | | | Diagnosis on admission—lobar pneumonia, malnutrition, secondary anemia. |
| | 1-6 | 40 | 3.55 | 6.7 | |
| | 1-7 | 40 | | | |
| | 1-8 | | 3.93 | 7.1 | Later developed pyelitis, otitis media, bronchopneumonia, and severe secondary anemia. |
| | 1-9 | 40 | | | |
| | 1-10 | 40 | | | |
| | 1-11 | 40 | 4.01 | 7.7 | |
| | 1-12 | 40 | | | At time of administration of $GeO_2$ scattered râles throughout chest, slight fever, moderate discharge from left ear. |
| | 1-13 | 40 | | | |
| | 1-15 | 40 | 4.00 | 7.7 | |
| | 1-19 | 40 | 3.84 | 7.5 | |
| | 1-20 | 40 | | | |
| | 1-22 | 40 | | | |
| | 1-23 | 40 | | | |
| | 1-24 | 40 | 4.03 | 8.0 | |
| | 1-25 | 40 | | | |

*Red blood count done before germanium was given.

†The average normal hemoglobin content for children is rather less than 14 grams per 100 c.c. of blood.

TABLE I—CONT'D.

EFFECT OF GERMANIUM DIOXIDE ON ERYTHROCYTES AND HEMOGLOBIN IN ANEMIA

| CASE | DATE 1922 | GERMANIUM DIOXIDE MG. | BLOOD ERYTHROCYTES MILLION PER C. MM. | HEMOGLOBIN GM. PER 100 C.C.† | REMARKS |
|---|---|---|---|---|---|
| | 1-26 | 40 | | | |
| | 1-27 | | 4.06 | 8.65 | |
| 5 L.F. | 7-28-22 | | 4.35 | 9.2 | Age 1¾ years. |
| | 7-29 | 30 | 4.38 | 9.2 | Diagnosis—rickets, malnutrition, secondary anemia. |
| | 7-31 | 50 | | | |
| | 8-1 | 50 | 4.33 | | |
| | 8-2 | 50 | | | |
| | 8-3 | 50 | | | |
| | 8-4 | | 4.32 | 10.0 | |
| 6. B.G. | 12-15-22 | | 3.32 | 7.9 | Age 6 years. |
| | 12-18 | 70 | | | Diagnosis—chronic endocarditis, double mitral disease, secondary anemia. |
| | 12-20 | 160 | | | |
| | 12-21 | 80 | 4.08 | 7.9 | |
| | 12-23 | 160 | | | |
| | 12-24 | 80 | 4.46 | 8.5 | |
| | 12-26 | 80 | 4.44 | 7.9 | |
| | 12-28 | 160 | 4.06 | 7.5 | |
| | 12-30 | 160 | 4.10 | 7.0 | |
| | 1-4 -23 | | 4.06 | 7.8 | |
| | 1-11 | | 4.39 | 8.1 | |
| | 1-17 | | 4.16 | 8.2 | |
| 7. V.L. | 12-18 | | 4.19 | 10.2 | Age 3 years. |
| | 12-27 | | 4.06 | 10.2 | Diagnosis—nephrosis, acute bronchitis. |
| | 12-28 | 80 | | | |
| | 12-29 | 80 | | | Red blood count and hemoglobin content stationary. |
| | 12-30 | 60 | 4.92 | 10.3 | |
| | 12-31 | 80 | 4.86 | | Urine contained large amount of albumin and large number of hyaline and granular casts. |
| | 1-2 | 80 | 4.86 | 10.8 | |
| | 1-3 | 80 | | | |
| | 1-4 | 80 | 4.68 | 11.0 | |
| | 1-5 | 60 | | | 1/14/23 developed bilateral otitis media. |
| | 1-6 | | 4.59 | 10.6 | |
| | 1-8 | | 4.16 | 10.1 | Diet—soft, meat- and salt-free. |
| | 1-11 | | 4.53 | 10.0 | |
| 8. J.R. | 11-6 | | 3.65 | 9.2 | Age 3½ years. |
| | 11-8 | 50 | | | Diagnosis—acute nephritis, otitis media. |
| | 11-9 | | | | |
| | 11-10 | 50 | 4.08 | 8.8 | Urine contained large amount of albumin, a large number of pus cells, and granular casts. |
| | 11-11 | 50 | | | |
| | 11-13 | 50 | 4.26 | 8.8 | |
| | 11-14 | 50 | | | |
| | 11-15 | | 3.85 | | Diet—salt-free and meat-free with exception of lemonade diet from November 11 to 15. |
| | 12-6 | | 3.60 | 8.3 | |
| | 12-7 | 70 | | | |
| | 12-8 | 70 | | | |
| | 12-9 | 70 | 3.90 | | |
| | 12-11 | 70 | | | |
| | 12-12 | 70 | 4.28 | 9.3 | |
| | 12-14 | 80 | | | |
| | 12-15 | 70 | | | |
| | 12-16 | | 4.29 | 9.2 | |
| | 12-19 | | 4.42 | 9.3 | |
| | 12-28 | | 3.95 | | |
| | 12-30 | 80 | | | |
| | 12-31 | 80 | | | |
| | 1-2 | 80 | 4.37 | 8.7 | |
| | 1-3 | 80 | | | |
| | 1-4 | 80 | 4.51 | 8.8 | |
| | 1-6 | | 4.59 | 8.8 | |

TABLE I—CONT'D.

EFFECT OF GERMANIUM DIOXIDE ON ERYTHROCYTES AND HEMOGLOBIN IN ANEMIA

| CASE | DATE 1922 | GERMANIUM DIOXIDE MG. | BLOOD ERYTHROCYTES MILLION PER C. MM. | HEMOGLOBIN GM. PER 100 C.C.† | REMARKS |
|---|---|---|---|---|---|
| 9. M.D. | 11-13 | 50 | 3.56* | 6.8 | Age 11 years. |
| | 11-15 | 140 | 4.04 | 7.0 | Diagnosis—Gaucher's dis- |
| | 11-17 | 140 | 3.64 | 7.1 | ease. |
| | 11-20 | 140 | 4.55 | 7.2 | |
| | 11-22 | 130 | 4.53 | 7.2 | |
| | 11-24 | 110 | 4.23 | 7.0 | |
| | 11-25 | 50 | | | |
| | 11-28 | | 4.21 | 7.0 | |
| | 12-1 | | 4.03 | 7.4 | |
| | 12-11 | | 4.08 | 7.3 | |
| | 12-21 | | 4.00 | 6.8 | |
| | 1-5 | | 4.48 | 6.7 | |
| | 2-8 | | 3.90 | | |
| 10 N.D. | 11-13 | 50 | 3.75* | 5.3 | Age 8 years. |
| | 11-15 | 140 | 3.90 | 5.9 | Diagnosis—Gaucher's dis- |
| | 11-17 | 140 | 4.07 | 6.1 | ease. |
| | 11-20 | 140 | 4.06 | 6.6 | |
| | 11-22 | 130 | 4.08 | 6.4 | |
| | 11-24 | 110 | 4.15 | 6.2 | |
| | 11-25 | 50 | | | |
| | 11-28 | | 4.15 | 6.7 | |
| | 12-1 | | 4.00 | 6.5 | |
| | 12-11 | | 3.76 | 6.4 | |
| | 12-21 | | 4.22 | 6.0 | |
| | 1-5 | | 4.20 | 6.0 | |
| | 2-8 | | 3.84 | | |
| 11. A.D. | 11-13 | 40 | 3.61* | 6.1 | Age 5 years. |
| | 11-15 | 100 | 3.66 | 6.5 | Diagnosis—Gaucher's dis- |
| | 11-17 | 100 | 3.73 | 6.7 | ease. |
| | 11-20 | 100 | 4.00 | 7.0 | |
| | 11-22 | 90 | 3.80 | 7.4 | |
| | 11-24 | 80 | 4.00 | 6.8 | |
| | 11-25 | 40 | 3.85 | 6.7 | |
| | 12-1 | | 3.88 | 6.2 | |
| | 12-11 | | 3.72 | 6.4 | |
| | 12-21 | | 4.12 | 6.9 | |
| | 1-5 | | 4.15 | 6.1 | |
| | 2-8 | | 3.90 | | |
| 12. I.D. | 11-20 | 30 | 4.27* | 7.1 | Age 3 years. |
| | 11-22 | 60 | | | Diagnosis—Gaucher's dis- |
| | 11-24 | 60 | 4.68 | 7.1 | ease. |
| | 11-26 | 60 | | | |
| | 11-28 | 30 | 4.00 | 7.3 | |
| | 11-30 | 60 | | | |
| | 12-1 | 50 | 4.40 | 7.7 | |
| | 12-2 | 50 | | | |
| | 12-11 | | 4.18 | 7.2 | |
| | 12-21 | | 4.40 | 7.9 | |
| | 1-5 | | 4.44 | 7.5 | |
| 13. N.I. | 1-22 | 40 | 2.90* | 4.7 | Age 6 months. |
| | 1-23 | 40 | | | Diagnosis—von Jacksch's |
| | 1-24 | 40 | | | anemia. |
| | 1-25 | 40 | 3.04 | | |
| | 1-26 | 40 | | | |
| | 1-27 | 40 | | | |
| | 1-29 | 40 | | | |
| | 1-30 | 40 | 2.89 | 4.7 | |

## REPORT OF CASES

Case 1.—A. K., male, aged one and one-half years, weighing twenty and one-half pounds, was admitted to the babies' ward June 29, 1922 with a history of cough and high fever one week previous to admission. His formula consisted of a mixture of equal parts of milk and water and one teaspoonful of sugar at a feeding. Occasionally oatmeal with milk was given. Physical examination: he was very pale, waxy colored, very poorly nourished, and appeared acutely ill; there was evidence of consolidation of the left lower lobe of the lung; the liver was palpable three finger breadths and the spleen two finger breadths below the costal margin; the ankle and wrist joints were slightly enlarged and the legs were slightly bowed. The temperature on admission was 103.5°F. the next day, rose to 101° F. for three days and then remained around 100° F. for the following week. Since July 11, the temperature has been practically normal. The red cell count on admission was 2,304,000 per c. mm., the hemoglobin 19 per cent (by Sahli). A second count two days later showed the red cells slightly less than 2,000,000; the hemoglobin remained unchanged. The urine was negative. Diagnosis: lobar pneumonia, malnutrition, rickets, severe secondary anemia.

Case 2.—P. F., female, aged two and one-half years, was admitted to the babies' ward June 9, 1922, with a history of right lobar pneumonia five weeks previous to entrance to the hospital, a left lobar pneumonia three weeks later. The temperature has been elevated since the onset, but has been of the septic type since shortly before admission. Physical examination: she was markedly undernourished and acutely ill; the chest presented signs of left sided empyema; the heart was displaced to the right; the liver was felt one finger's breadth below the costal margin. A thoracotomy was performed June 13, 1922. The temperature remained intermittent until July 20; since that time it has been normal with an occasional rise to 99° to 100°F. There was a profuse purulent discharge from the pleural cavity. The red blood count, July 18, was 3,552,000 and the hemoglobin 24 per cent (by Sahli). The urine contained small amounts of pus on several occasions. Diagnosis: empyema, pyelitis and secondary anemia.

Case 3.—D. R., male, aged one and one-half years, was admitted November 18, 1922 with a history of a cough and slight fever for one week. Physical examination showed a very well developed and nourished child with signs of pleurisy with small amount of effusion of the left chest. The temperature on admission was 102°F., but reached normal the second day with an occasional rise until his discharge, December 24, 1922.

Case 4.—E. F., female, aged six months, came to the babies' ward on November 12, 1922, with a history of diarrhea, projectile vomiting, and slight fever for one day. Physical examination: she was rather poorly developed and nourished; the lungs showed evidence of a pneumonia of the right middle lobe which was later complicated by marked malnutrition. During her stay in the hospital she developed pyelitis, left otitis media, bronchopneumonia, and severe secondary anemia. The red blood count on admission was 3,900,000, hemoglobin 50 per cent (by Sahli); just before germanium therapy, 3,110,000, hemoglobin 6.7 gm.

Case 5.—L. F., male, aged one and three-fourths years, was admitted July 22, 1922 with a diagnosis of severe rickets, malnutrition, and secondary anemia.

Case 6.—B. G., male, aged six years, was diagnosed as chronic endocarditis, double mitral disease, and secondary anemia. There was myocardial insufficiency but no edema.

Case 7.—V. L., female, colored, aged three years, weighing twenty-seven pounds, was admitted July 24, 1922, with diagnosis of nephrosis and bronchitis. History: swelling of face and extremities one month previous to admission following an attack of acute bronchitis. Physical examination: child was well developed and nourished; slight puffiness of face and eyelids and slight pitting edema of ankles was present; there were numerous sibilant and sonorous râles throughout the chest. On October 18, abdominal tap was done and 1200 c.c. clear fluid obtained. The anasarca gradually cleared up. The urine

contained a large amount of albumin, a large number of hyaline and granular casts. The phthalein elimination in two hours was 16 per cent. The chemical blood: urea 21.1, uric acid 3.2, mg. per 100 c.c., cholesterol 0.436, and chlorides 0.513 per cent.

Case 8.—J. R., male, aged three and one half years, was admitted November 3, 1922. Five weeks previous to admission he had a "heavy cold," followed by otitis media one week later. Blood appeared in the urine and slight edema of the ankles and eyelids was noted three days before entrance to hospital. Physical examination showed no abnormalities except for a left otitis media; no edema of the eyelids or extremities. Chemical blood: urea 26.9, uric acid 3.7 mg. per 100 c.c., chlorides 0.60, sugar 0.14 per cent. Phenolsulphonephthalein elimination in two hours 37.2 per cent. Red cell count December 4: 3,784,000, hemoglobin 65 per cent (by Sahli). Urine examination: large amount of albumin, many pus cells and granular casts. Chemical blood December 19: urea 15.9, and uric acid 2.7 mg. per 100 c.c., chlorides 0.538, sugar 0.098 per cent. Urine: negative. His diet was a low protein, salt-free diet. Discharged December 19, 1922. Diagnosis: acute nephritis, otitis media. He was readmitted seven days later complaining of pain in the left ear and frequent urination. He was discharged January 6, 1923, feeling well.

Case 9.—M. D., male, aged, eleven years.

Case 10.—N. D., male, aged eight years.

Case 11.—A. D., male, aged five years.

Case 12.—I. D., female, aged three years.

The above four cases, 9, 10 and 12, members of the same family, and case 11, a cousin, represent cases of a chronic type of familial disease, possibly Gaucher's Disease. When first seen, several years ago, the anemia was very marked, and symptoms of weakness, faintness and exhaustion were present. The liver and spleen in each of the cases were markedly enlarged. Following x-ray treatment to the spleen and blood transfusions there was a decided improvement in their condition. No therapy of any kind had been administered one year previous to the trial with germanium. At the time of germanium therapy the anemias had reached a stationary period.

Case 13.—N. I., male, aged six months, was admitted to the babies' ward December 1, 1922, with a diagnosis of malnutrition and von Jaksch's anemia. He received a blood transfusion of 180 c.c. on December 7. The blood count preceding the transfusion was lost. Since the transfusion the red blood counts were as follows: December 8—4,224,000, hemoglobin 9.2 gm. per 100 c.c. December 14—4,096,000, hemoglobin 9.1 gm. December 29—4,248,000, hemoglobin 8.4 gm. January 22—2,900,000, hemoglobin 4.7 gm. March 9—846,000.

## DISCUSSION

The data on the red cell counts, hemoglobin, and amounts of germanium dioxide administered in each case are recorded in Table I. A brief history of the cases is also given.

Cases 1, 2, 3, and 4 represent cases of anemia following acute infections. That the infection was the sole cause of the anemia is quite improbable, since in Cases 1, 2, and 3 there was also a history of marked malnutrition, a condition so often associated with the secondary anemias in childhood. It will be noted that in each of these cases a very prompt and sustained increase in the number of erythrocytes and in the amount of hemoglobin was obtained (Table I and Charts 1, 2, and 3). The initial rise in red cells occurred from two to seven days after the first dose was administered, and the maximum increase was obtained in from ten to twenty days. The amount of germanium dioxide required to effect this initial rise was comparatively

small, for in Cases 3 and 4, two doses, in Case 1, three doses, and in Case 2, five doses had been given. It is observed that, as a rule, the red cell counts remained fairly stationary during the second and third week of germanium therapy, and that further administration of this compound had little or no further effect on the number of erythrocytes.

The initial rise in the hemoglobin content was also quite prompt, although generally more delayed. The increase in hemoglobin took place more gradually, but continued even after the red cell count had become stationary. In these observations also, as in the experiments reported by Hammett and Müller, the response to the germanium appeared to be greater in those cases with a low initial count. There was no tendency for the red cells or the hemoglobin to return to their previous levels after the treatment was dis-

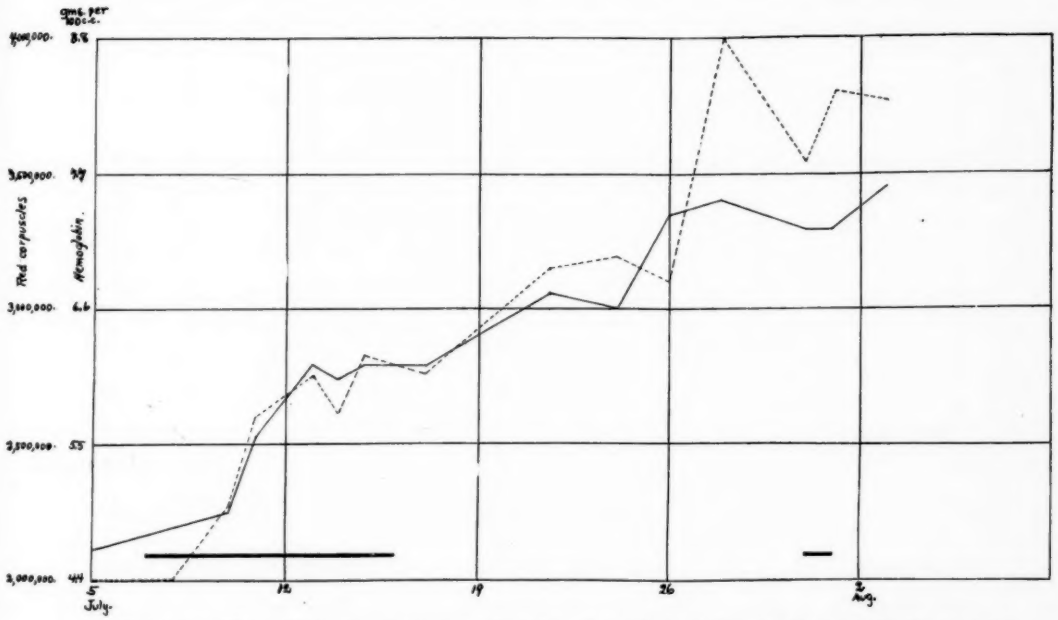


Chart 1.—The effect of germanium dioxide on erythrocytes and hemoglobin in a case of convalescing lobar pneumonia, malnutrition, rickets, and severe secondary anemia (Case 1). The broken line represents the red corpuscles, the light line the hemoglobin, and the heavy line the period of germanium therapy.

continued. Aside from the remarkable results noted in the case of the blood, some clinical improvement also manifested itself. By clinical improvement we mean increased appetite, return of pink color to skin, brighter expression, and greater activeness. This was particularly apparent in Cases 1 and 4.

In a case of rickets (Case 5) no increase in the red cells occurred within seven days after the first dose was given; however, a slight rise in hemoglobin was noted. Unfortunately this case was observed for only one week.

Case 6, diagnosed chronic cardiovalvular disease, double mitral disease, and secondary anemia, showed a notable increase in erythrocytes three days after the germanium was administered; however, the hemoglobin was still at the original level. Three days later another rise in the red cell count

was observed with a rise in hemoglobin of 0.6 gm. Additional doses of germanium had no further effect on either the erythrocytes or hemoglobin. Ten days after the initial dose the red cell count reached a constant, at which level it remained eighteen days after the discontinuation of the compound. The hemoglobin showed greater fluctuations, but only a slight tendency toward an increase over the control. The prompt and sustained rise in the red cells noted in this case can hardly be explained on the basis of a normal regeneration, for normally a rise of almost one million in three days is never encountered unless there is a change in plasma volume which certainly is improbable in this case since the red blood count remained constant for a period of twenty days.

A case of nephrosis (Case 7) showed a rather fixed red cell count and hemoglobin content for at least ten days before germanium was given. No

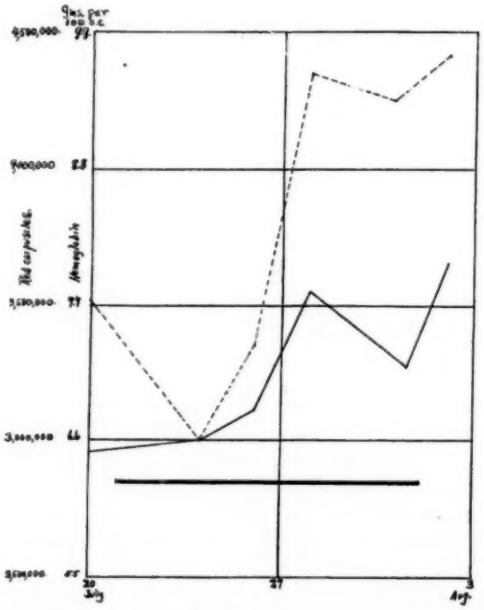


Chart 2.—The response to germanium by increase in red blood cells and hemoglobin in a case of empyema, pyelitis, and secondary anemia (Case 2). The broken line represents the red cells, the light line the hemoglobin, and the heavy line the period of germanium therapy.

edema was present during the period of observation. Two days following the initial dose an increase in red cells of nine hundred thousand per c.mm., the highest point reached, was observed. The hemoglobin content increased gradually and reached its maximum increase four days later. Immediately after the germanium was discontinued, the hemoglobin began to drop to its original level. The red cell count also showed a decrease, but was still 400,000 above the control counts. Case 8, a case of acute nephritis and otitis media received germanium dioxide over three different periods. A definite increase in red cells was obtained in each period after two to three doses had been given, but as soon as the germanium was discontinued, the red cell counts dropped suddenly. During the first period the hemoglobin showed a

slight decrease, during the second period an increase to the original level, and another drop during the last period.

In the last two cases (7 and 8) only a temporary improvement in the blood constituents could be expected, for the etiologic factors were still present. At the time the observations were made both patients were on a low protein diet and there were still excessive losses of the albuminous materials of the blood through the kidneys.

The cases of Gaucher's disease (9, 10, 11 and 12), members of the same family, responded readily to germanium by increases in the number of erythrocytes and in the amount of hemoglobin. Two months after the treatment was discontinued the red cell count in each case was still above the controls by from 200,000 to 400,000. The hemoglobin, however, had returned to prac-

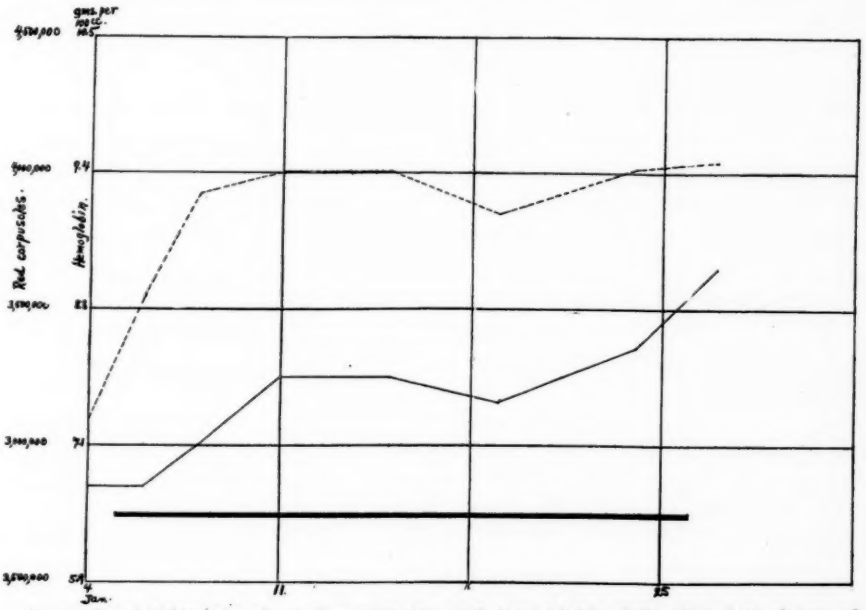


Chart 3.—Sudden rise in red corpuscles and hemoglobin following first doses of germanium in a case of pneumonia, malnutrition, otitis media, and severe secondary anemia (Case 4). The broken line represents the red cells, the light line the hemoglobin, and the heavy line the period of germanium therapy.

tically the original level after about six weeks in Cases 9 and 11, but was still above the controls in Cases 10 and 12.

In a case of von Jaksch's anemia no changes were noted in either the red cell counts or in the hemoglobin content following germanium administration. No better results were obtained with a blood transfusion with the exception of a temporary improvement in the red cell count and hemoglobin due to the extra amount of blood supplied. Arsenic and iron, and radium to the long bones likewise produced no results. In anemias of this type where blood destruction takes place with increased rapidity, and the erythroblastic functions are strained to their utmost, no improvement can be expected until the etiologic factor is removed.

We feel justified in our conclusion that the rise in the red blood cells and in the hemoglobin content in the cases here presented were due to the

action of germanium. We must remember in comparing the results obtained in the normal with those obtained in the anemias, that in the latter the etiologic factor may still be present to interfere with the regeneration of erythrocytes. The best results were obtained in the anemias associated with malnutrition and acute infections as evidenced by the marked and sustained increase in the number of erythrocytes and hemoglobin, and by the noticeable clinical improvement.

We have no data at the present time to prove that germanium is of any value in the eradication of the various etiologic factors. For this reason it should not be given with the hope of improving all anemias, but should rather be looked upon as a very helpful agent in stimulating normal erythrocyte formation. Iron in conjunction with germanium may have a more decided influence on the hemoglobin than germanium alone.

## SUMMARY

The therapeutic action of germanium dioxide was studied in thirteen unselected cases of anemia in childhood; five cases of anemia following malnutrition and acute infections, two cases accompanying acute nephritis, one case of chronic cardiovalvular disease, four cases of Gaucher's splenomegaly, and one case of von Jaksch's anemia. All the cases, with the exception of the case of von Jaksch's anemia, showed a definite response by marked and sustained increases in the number of erythrocytes and in the hemoglobin content. The most notable results were obtained in the cases of anemia following malnutrition and acute infections. Some clinical improvement was evident in a number of cases.

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# PRELIMINARY NOTE ON THE DIFFERENCE IN PHARMACOLOGIC ACTION OF POTASSIUM AND OF SODIUM SALTS WHEN ADMINISTERED INTRAVENOUSLY*

By David Loeser and A. Lincoln Konwiser, New York City

DESPITE the fact that sodium and potassium are divergent in their physiologic effects, in practical therapeutics their iodides have been considered interchangeable when given by mouth. We have found, however, that when introduced intravenously there is a marked difference in the toxicity of potassium iodide and of sodium iodide. Extending the study further, we noted the same results with potassium and sodium bicarbonates. It is the purpose of this preliminary note to present a series of experiments which we believe indicate clearly that potassium salts are more toxic intravenously than the corresponding sodium salts. These experiments point out, moreover, that the results of the oral administration of a remedy cannot be transferred bodily to intravenous administration without a careful preliminary study.

## EXPERIMENTAL

Injections were made in young white rats. They were weighed and marked for indentification with a saturated solution of phenylendiamine to which had been added an equal volume of hydrogen peroxide. This dye soon sets to a deep black which lasts several months.

Table I

Toxicity Tests with KI.

| NUMBER RATS | KI/KILO GRAMS | K/KILO GRAMS | I/KILO GRAMS | NUMBER TIMES THERAPEUTIC DOSE | REMARKS |
|---|---|---|---|---|---|
| 1 | 0.086 | 0.0201 | 0.065 | 3 | Survived |
| 1 | 0.114 | 0.0268 | 0.087 | 4 | Survived |
| 1 | **0.143** | **0.0336** | **0.109** | **5** | Died on table |
| 1 | 0.151 | 0.0355 | 0.115 | 5.25 | Died on table |

Therapeutic dose is considered to be 20 c.c. of a 10 per cent solution, i.e., 2 gm.

The figures in heavy type throughout the tables represent the range of the minimum lethal dose. Inasmuch as our experiments are comparative, we have not attempted to determine the absolute minimum lethal dose.

Each rat is tied down to an operating board (see B. G. Roth, U. S. Public Health Reports, April 1, 1921) and the solution to be administered is injected intravenously into the right or left saphenous vein. Before making the incision, the hair is shaved off the legs. For this purpose we employ a Gillette razor in a Mark Cross razor holder, which makes a splendid combination; it is much less liable to cut the skin than an ordinary razor. Again, hairs

*From the New York Intravenous Laboratory.
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block up the safety grid of an ordinary safety razor. Otherwise our technic is similar to that employed by Roth in his official method for testing arsphenamine and allied compounds. We use a burette and make the injection by gravity with a No. 23 or 24 B. & S. gauge needle, one inch long.

The results obtained are shown in the following tables. The figures in the first column indicate the number of rats injected at each concentration. The figures in the second column give the actual amount in grams per kilo weight of each salt injected. From these figures we have calculated those in the third and fourth columns.

In the fifth column we have adopted as the therapeutic dose, that dose of the sodium salt which has been reported most frequently, both in the literature and in personal communications, as giving the most satisfactory results in intravenous medication. For purposes of comparison we have adopted the same standard for the potassium salts.

TABLE II

TOXICITY TESTS WITH NaI

| NUMBER RATS | NaI/KILO GRAMS | Na/KILO GRAMS | I/KILO GRAMS | NUMBER TIMES THERAPEUTIC DOSE | REMARKS |
|---|---|---|---|---|---|
| 1 | 0.71 | 0.109 | 0.593 | 25 | Survived. |
| 1 | 0.857 | 0.131 | 0.725 | 30 | Died after 24 hours. |
| 5 | 1.00 | 0.153 | 0.847 | 35 | All survived. |
| 6 | **1.14** | **0.175** | **0.965** | **40** | Two, or 33 1/3% survived. Others died at intervals of from twelve hours to nine days. |
| 6 | 1.28 | 0.196 | 1.083 | 45 | Four, or 66 2/3% survived. Others died within 42 hours. |
| 2 | 1.429 | 0.219 | 1.209 | 50 | Both died, one in 24 hours other in 36 hours. |
| 1 | 2.143 | 0.329 | 1.813 | 75 | Died in 24 hours. |
| 1 | 2.859 | 0.439 | 2.419 | 100 | Died in 12 hours. |

Therapeutic dose is considered to be 20 c.c. of a 10 per cent solution, i.e., 2 gm.

TABLE III

TOXICITY TESTS WITH $KHCO_3$

| NUMBER RATS | $KHCO_3$/KILO GRAMS | K/KILO GRAMS | $HCO_3$/KILO GRAMS | NUMBER TIMES THERAPEUTIC DOSE | REMARKS |
|---|---|---|---|---|---|
| 3 | 0.10 | 0.039 | 0.061 | 7.0 | Two survived, 1 died on table. |
| 2 | 0.11 | 0.043 | 0.067 | 7.5 | Both survived (1 almost died, however). |
| 1 | 0.120 | 0.047 | 0.073 | 8.5 | Almost died, but survived. |
| 2 | **0.125** | **0.049** | **0.076** | **8.8** | One survived, though almost lifeless; other died. |
| 3 | 0.141 | 0.055 | 0.086 | 10.0 | Two died on table, other survived. |

Therapeutic dose is considered to be 20 c.c. of a 5 per cent solution, i.e., 1 gm.

## DISCUSSION AND SUMMARY

The above figures show not only that potassium iodide has a comparatively high degree of toxicity, but also that potassium is far more toxic intravenously than sodium. This is shown more clearly in Table VI, in which the minimum lethal doses have been tabulated and contrasted.

It can be seen that whereas 0.175 grams of sodium per kilo weight are tolerated, only 0.034 grams per kilo weight are tolerated in the case of potassium when given in the form of potassium iodide. The difference is even more marked in the case of the bicarbonates. The fact that more of the anion per kilo body weight is given when sodium salts are administered than when potassium salts are administered, indicates that the toxicity of the anion does not materially influence the results.

TABLE IV

TOXICITY TESTS WITH $NaHCO_3$

| NUMBER RATS | $NaHCO_3$/KILO GRAMS | Na/KILO GRAMS | HCO/KILO GRAMS | NUMBER TIMES THERAPEUTIC DOSE | REMARKS |
|---|---|---|---|---|---|
| 1 | 0.357 | 0.098 | 0.259 | 25 | Survived |
| 1 | 0.714 | 0.274 | 0.518 | 50 | Survived |
| 1 | 1.071 | 0.293 | 0.778 | 75 | Survived |
| 1 | 1.428 | 0.391 | 1.007 | 100 | Survived |

Therapeutic dose is considered to be 20 c.c. of a 5 per cent solution, i.e., 1 gm.

TABLE V

TOXICITY TESTS WITH $KNO_3$

| NUMBER RATS | $KNO_3$/KILO GRAMS | K/KILO GRAMS | $NO_3$/KILO GRAMS | NUMBER TIMES THERAPEUTIC DOSE | REMARKS |
|---|---|---|---|---|---|
| 2 | 0.114 | 0.044 | 0.027 | 25 | Very sick |
| 1 | 0.160 | 0.062 | 0.038 | 35 | Died in 2 minutes |
| 1 | 0.228 | 0.088 | 0.042 | 50 | Died in 2 minutes |

Tentative therapeutic dose is considered to be 0.32 gm. contained in 5 c.c. solution.

TABLE VI

COMPARISON OF RESULTS

| SALT USED | CATION/KILO AT MINIMUM LETHAL DOSE GRAMS | ANION/KILO AT MINIMUM LETHAL DOSE GRAMS | FACTOR OF SAFETY |
|---|---|---|---|
| KI | 0.034 | 0.109 | 5 |
| NaI | 0.175 | 0.965 | 40 |
| $KHCO_3$ | 0.049 | 0.076 | 8.8 |
| $NaHCO_3$ | > 0.391 | > 1.007 | > 100 |
| $KNO_3$ | 0.062 | 0.038 | 35 |

From the experiments of other investigators we may infer that this increased toxicity of the potassium salts is due to a depression of the heart and central nervous system, brought about by the potassium ion when introduced directly into the blood stream. Heinekamp[1] has shown that potassium salts influence the cardio-inhibitory center of the terrapin, producing inhibition of the heart when introduced intravenously. Hering[2] demonstrated similar effects in the dog, and, to a lesser extent, in the rabbit. Slow, continuous intravenous infusions of small doses of KCl resulted in central vagus stimulation. Larger doses caused ventricular and eventually auricular fibrillation. Extrasystolic tachycardia also occurred. Working with cats, Mathison[3] obtained the same results. When injected intravenously, potassium salts bring about a primary fall of blood pressure due to dilatation and diminished

output of the heart, immediate stoppage in diastole, marked sinus slowing, partial or complete heart block, ventricular fibrillation with auricular contractions.

This series of experiments again points to the marked difference between the pharmacologic action of a drug when given by mouth and when injected intravenously. It is well known that 5 grams of potassium iodide per dose are easily tolerated when given by mouth, yet this dose would probably prove fatal if introduced intravenously. The contention that sodium and potassium iodides are interchangeable, therefore, does not seem to be borne out by experimental fact. The following statement by Sollman[4] offers one explanation of the discrepancy: "The effect of potassium, as of other cations, varies greatly according to the channel of introduction, because the excretory capacity for these ions is usually much greater than the rate of their absorption from the alimentary canal. Oral administration is, therefore, effective only if very large doses are given, if the mucous membranes are corroded, or if the excretion is checked, as by ligation of the kidneys."

Another explanation, however, is that the intravenous method offers a far more delicate technic for the study of the intrinsic pharmacologic action of a drug than administration by mouth. In the latter method so many intervening reactions have occurred during the passage of the drug through the gastrointestinal tract that by the time the drug has entered the blood it is impossible to determine with any degree of accuracy the limiting factors. In the intravenous method, however, we are starting out with the results desired, i.e., the presence of the drug in the blood stream, and we can thus study its pharmacology, feeling confident that our conclusions are not vitiated by indirect actions which we have no means of studying or controlling.

In this connection it is interesting to note that as far back as 1914 Macht[5] pointed out that when sodium iodide and potassium iodide were injected into dogs, a decided difference in the action of the two drugs was noted. He states, "The action of Na and K ions on the heart and vessels can best be understood from the action of their component factors. NaI possesses no depressing property, for the Na ion is a vascular constrictor and a cardiac stimulant, and the I ion, insofar as it is free to act, has the same action.

"KI, on the other hand, clearly shows the depressing effect of the K ion on the heart and vessels, especially of mammals, not only on isolated organs but also in the living animals. It is, therefore, not a matter of indifference which of the iodides is to be chosen for the purpose of depressing the circulation, as for instance in the case of aneurism. The above pharmacological analysis of the action of the iodides, furthermore, shows that as far as experimental evidence goes, the iodides show no special virtue of lowering blood pressure, but that effect is due entirely to the K, and could be produced even more efficiently by other K salts." Other experimental evidence proves, however, that Macht's conclusions are not entirely valid. Iodides in themselves are effective in hypertension probably through their effect in stimulating the elimination of nitrogen compounds.

Translated into practical therapeutics, our experiments indicate that

when the iodide effect only is desired, sodium iodide should ordinarily be chosen for intravenous administration. Osborne[6] has recently shown that, when administered by mouth, potassium iodide seems to be utilized by the body in the form of sodium iodide. Only a slight amount of the potassium was absorbed. It would seem that the intravenous route is far more direct and satisfactory than the alimentary canal route if one uses as his criterion the speed and efficiency with which a drug is utilized, and the accuracy with which it may be administered and controlled.

The fact that potassium is comparatively toxic when introduced into the vein suggests that we may have a very valuable therapeutic agent in potassium, once its action is fully understood. That the toxic symptoms are not the result of the mode of administration, but rather an expression of the pharmacologic action of the drug, is shown by the fact that the same symptoms are elicited when a sufficiently large dose is given by mouth.[4] The work of Mathison, and of Macht, referred to above, points out that potassium should be decidedly useful in such conditions as hypertension. Moreover, in view of the fact that many of the newer cancer theories favor the substitution of potassium for sodium as far as possible, clinical investigation on the action of potassium intravenously should prove of great value and interest.

## CONCLUSIONS

1. Potassium salts are far more toxic intravenously than the corresponding sodium salts.

2. The action of a drug intravenously must be carefully studied; it cannot be inferred invariably from its action when administered by mouth.

3. When the effect of the iodide radical is desired, sodium iodide, rather than potassium iodide, should be used intravenously. When the potassium effect is desired, potassium iodide is naturally chosen. In that event the dosage must be carefully regulated.

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# *LABORATORY METHODS*

## THE MEASURE OF URINARY SUGAR FOLLOWING THE INGESTION OF A STANDARD MIXED CARBOHYDRATE BREAKFAST*

By Herman Sharlit, M.D., and William G. Lyle, M.D., New York, N. Y.

WITH the introduction of accurate methods for the determination of the carbohydrates in normal urine, interest has been renewed in the study of carbohydrate metabolism in the nondiabetic from the point of view of urinary findings. Greenwald[1] endeavored to investigate the nature of the carbohydrates of normal urine. He was inclined to conclude that possibly one-third of all the sugars present were pentoses, and offered the suggestion that the pentoses might be derived from protein.

By means of these newer methods much data has already been presented, and many conclusions drawn therefrom. Benedict and Osterberg[2, 3] and Folin and Berglund[4] have been the chief investigators in this field. These pairs of investigators, however, have in many instances placed diametrically opposite interpretations on their data, and have quite vigorously disagreed over what we may rightly consider basic facts in this field of study.

We shall not review the details of their agreements and disagreements, but limit ourselves to stating the observations that led to our study. Following the ingestion of food the hourly rate of excretion of normal urinary sugar increases over that of the fasting rate. To this increase Benedict and Osterberg applied the term glycuresis. Glycuresis ensues following any diet, even if free of carbohydrate. Both groups of observers concur in these findings. Folin and Berglund, however, hold glycuresis as representing the excretion of foreign unassimilable carbohydrates, while Benedict and Osterberg maintain that it is indicative of failure of the complete utilization of sugar. This latter interpretation places all persons in the class of the diabetic, making the normal individual differ from the diabetic but in degree, provided it can be demonstrated that normal urinary sugar either contains "diabetic sugar," glucose, or can be derived from the administration of glucose.

We undertook to secure a clinical test for carbohydrate tolerance based upon the quantitative output of sugar following a test breakfast. If, as Folin and Berglund suggested, the degree of glycuresis was dependent upon the amount of nonassimilable carbohydrate in the food, our findings would be controlled in largest measure, by the character of the carbohydrates in

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the test breakfast. For this reason we gave as a test breakfast one containing a mixture of the usual table carbohydrates, including some partially digested carbohydrate (grape-nuts) which these investigators suggested as a likely source of the nonassimilable sugar. The breakfast contained between 110 and 125 gm. of carbohydrate, consisting of a glass of milk, vienna roll, 40 gm. sucrose and an individual service package of grape-nuts (about 45 gm.). To this was added another glass of fluid; either tea, coffee or water. The subject was called upon to refrain from partaking of food or drink following the evening meal before the test breakfast. He voided on retiring and passed a control sample in the morning before the test. Two hours after the test breakfast sample No. 2 was voided. The subject then drank a glass of water and 2 hours later, i.e., 4 hours after the breakfast the third and last sample was collected. The total quantity of sugar in each sample was estimated by the Benedict-Osterberg[5] picric acid-acetone method. As a time saving measure we eliminated the filtration step dispensing with clarifying the urine with bone black.

At the same time we sought to throw some further light on the suggestion of Greenwald that the normal urinary sugar may be in some way related to protein metabolism by estimating the nitrogen concentration of each sample and correlating this concentration with that of the sugar in the same specimen. The correlation was axpressed as a percentage

$$\frac{\text{wt. of carbohydrate per c.c.}}{\text{wt. of nitrogen per c.c.}} \times 100$$

A few preliminary experiments gave such astonishing results relative to this carbohydrate nitrogen ratio that we considered it all important to emphasize this feature of the findings in all the subsequent experiments. In Table I are given a few samples of our preliminary experiments. Therein are recorded the findings after six different breakfasts with subject Gwd. Following 100 gm. of dextrose the total carbohydrate elimination in 4 hours was 126 mgm., while following 100 gm. of protein the excretion in a like number of hours was 224 mgm. However, the carbohydrate nitrogen ratio was almost identical in both instances. By increasing the amount of carbohydrate in the breakfast the carbohydrate nitrogen ratio values could be forced up as is shown by the succeeding records of subject Gwd. Subject L. also shows remarkable constancy in his carbohydrate-nitrogen ratio figures following two distinctly different diets in spite of the very significant difference in the total sugar elimination or difference in period of observation following the intake of food. Subject O gives similar findings emphasizing the constancy of the carbohydrate-nitrogen ratio following dissimilar diets.

In Table II are given the findings with presumably normal subjects after the administration of our mixed carbohydrate test breakfast (Spec. 1 control sample before breakfast; specimen 2 elimination two hours after breakfast; specimen 3 elimination 4 hours after breakfast). The total carbohydrate elimination for the 4 hours after the test varies as much as 300 per cent, the

values ranging from 110 mgm. to 348 mgm. The carbohydrate nitrogen ratios, on the other hand, swing between much narrower limits and their numerical values are quite independent of the total carbohydrate elimination. Almost all the ratio in the specimens following the test breakfast keep below 11 per cent.

It would be profitable to take stock at this point, of the suggestions offered by the data of Tables I and II. We should at the very outset distinctly distinguish between carbohydrate intolerance and diabetes. All diabetics are intolerant of carbohydrates to greater or less degree; but there

TABLE I

CARBOHYDRATE AND NITROGEN ELIMINATION FOLLOWING SPECIAL MEALS

| SUBJECT | VOL. URINE C.C. | HOURS OF ELIMINATION | NITROGEN TOTAL GM. | NITROGEN PER C.C MGM. | CARBOHYDRATES TOTAL GM. | CARBOHYDRATES PER C.C MGM. | CARBOHYDRATE NITROGEN RATIO % | CHARACTER OF MEAL |
|---|---|---|---|---|---|---|---|---|
| Gwd. | 90 | 4 | 2.26 | 25.17 | 126 | 1.4 | 5.6 | 100 gm. dextrose. |
| " | 288 | 4 | 4.125 | 14.3 | 224 | 0.78 | 5.4 | 100 gm. protein (white of eggs and cheese). |
| " | 250 | 3 | 3.50 | 14.2 | 210 | 0.84 | 5.9 | Baked beans, 2 rolls, butter milk and cherry pie. |
| " | 400 | 4 | 2.85 | 7.13 | 231 | 0.578 | 8.1 | One-half cantaloupe, ½ lb. cherries, 6 plums, roll, 2 oz. blackberry jelly. |
| " | 144 | 4 | 2.00 | 13.88 | 199 | 1.38 | 10.0 | Two baked apples, 6 plums, 2 peaches, roll, apple jelly, 1½ oz. raisins. |
| " | 200 | 4 | 2.20 | 10.99 | 210 | 1.05 | 9.6 | 50 gms. grapenuts, 20 gm. sucrose, glass milk and roll, (mixed carbohydrate test meal). |
| L. | 460 | 12 | 7.50 | 16.31 | 470 | 1.02 | 6.2 | Supper of ½ lb. cheese and three chops. |
| " | 118 | 4 | 1.99 | 16.86 | 126 | 1.07 | 6.3 | Three slices toast, 4 lumps sugar, 2 ounces marmalade. |
| O. | 155 | 4 | 2.42 | 15.64 | 127 | 0.818 | 5.2 | One-half lb. cheese, 6 whole eggs. |
| " | 218 | 4 | 3.16 | 14.53 | 172 | 0.79 | 5.4 | Fish, mashed potatoes 2 glasses milk, huckleberry pie. |

exists a large group of persons not diabetics who have been adjudged sugar intolerants by reason of their failure to pass a satisfactory tolerance test of one kind or another. Of this latter group only those can be placed in the "early diabetic" or "prediabetic" category whose intolerance can be shown as due to a failure to properly burn glucose. Since the test herein presented does not possess the virtue of detecting intolerance due to a disturbed glucose metabolism alone, it can only serve the purposes of a "diabetic test" to the extent that all other tests based on urine examination do. We must, however, not overlook the possibility or rather the probability of the existence of disturbances of carbohydrate metabolism that do not center about glucose or any of the other specific sugars already identified and clinically catalogued. Removing the focus of our interest

from glucose to the unknown mixture of saccharides found in all urines gives a different, and, we believe, as equally important, approach to the proper study of the data secured by the test breakfast.

Little is as yet known concerning the derivation of the sugars in normal urine, nor anything of the chemical nature of these sugars. We have touched but slightly upon the conflicting judgments of Benedict and Osterberg and Folin and Berglund and have further complicated the problem by introducing data based on the suggestion of Greenwald that some of the carbohydrates may be of protein origin. Unfortunately we are not in possession of evidence that would be considered as having a direct bearing on any of the moot points in the problem. We can, we believe, throw the best light on the probable meaning of our findings by simply confining ourselves to a study of the quantitative output of the mixed sugars as correlated with carbohydrate intake and nitrogen excretion.

Table I contains a forceful suggestion that the normal urinary sugar may be in some way bound up with protein metabolism. We base this statement on the quite constant values of the carbohydrate-nitrogen ratio on such widely dissimilar diets as 100 gm. protein and 100 gm. carbohydrate. That all the normal urinary sugar is not of protein origin is quite certain; for if protein alone were the source, the carbohydrate ratio would be a constant (the rates of excretion being equal) at all times and on all diets provided only the carbohydrate intake was within the limit of the subject's tolerance. Greenwald after days of exclusive high protein feeding to a dog depressed the carbohydrate-nitrogen ratio to one per cent. So, too, can one by high carbohydrate feeding increase that ratio to several hundred per cent; that is to say, induce a true glycosuria. We must take it as most plausible that the factors determining the ratio are (1) protein of the diet, (2) carbohydrate of the diet and (3) peroid of observation, since the rates of nitrogen and carbohydrate excretion differ. Making all these three factors constant, as we do in feeding a fixed test breakfast and collecting urine samples at stated intervals thereafter, supply the conditions under which the carbohydrate-nitrogen ratio should tend to vary but slightly. A study of Table II—specimens after the test breakfast—give quite constant values for the carbohydrate-nitrogen ratio. On the other hand, in spite of the fixed amount of carbohydrate and protein in the breakfast the absolute measures of sugar and nitrogen in the urine samples of the different subjects vary markedly. We are at present not prepared to offer any explanation for the greater constancy of the carbohydrate-nitrogen ratios as against the absolute amounts of excreted sugar after the test breakfast.

Can, however, the carbohydrate-nitrogen ratio serve to gauge the degree of sugar intolerance after a test meal? It has been the custom to estimate sugar tolerance on the examination of urine excreted after a test diet by simply determining the presence or absence of a qualitative test for sugar in the urine examined. This simply means that a test is made to determine whether or not the specimen contains a concentration of sugar greater than 0.2 per cent. Tolerance is determined on the basis of a mere concentration

test. That such a procedure can give but the crudest of results needs little discussion. With methods available for estimating the smallest amount of urinary sugar interest will necessarily shift to measuring the absolute quantities of sugar excreted.

The data in Tables I and II not only give information concerning absolute amounts of excreted sugar but introduce the old idea of the concen-

TABLE II

MIXED CARBOHYDRATE TEST MEAL

Individual service package grapenuts (45 gm.); 40 sucrose, glass of milk and roll. (Total between 100 and 125 gm. carbohydrate)

| SUBJECT | SPECIMEN NUMBER | VOL. URINE | NITROGEN | | CARBOHYDRATES | | CARBOHYDRATES NITROGEN RATIO |
|---|---|---|---|---|---|---|---|
| | | C.C. | TOTAL GM. | PER C.C. MGM. | TOTAL MGM. | PER C.C. MGM. | % |
| N.O. | 1 | 260 | 3.95 | 15.21 | 260 | 1.00 | 6.6 |
| " " | 2 | 140 | 1.83 | 13.09 | 182 | 1.30 | 9.9 |
| " " | 3 | 78 | .898 | 11.53 | 87 | 1.11 | 9.7 |
| Grs. | 1 | 156 | 2.46 | 15.79 | 215 | 1.38 | 8.7 |
| " | 2 | 115 | 1.15 | 9.99 | 93 | 0.807 | 8.1 |
| " | 3 | 350 | 1.03 | 2.95 | 79 | 0.227 | 7.7 |
| Lfy. | 1 | 260 | 1.90 | 7.31 | 135 | 0.52 | 7.1 |
| " | 2 | 142 | 1.31 | 9.24 | 197 | 1.39 | 15.0 |
| " | 3 | 130 | 1.02 | 7.82 | 101 | 0.78 | 10.0 |
| Beruf | 1 | 378 | 2.86 | 7.56 | 250 | 0.66 | 8.7 |
| " | 2 | 142 | 1.29 | 9.06 | 145 | 1.02 | 11.2 |
| " | 3 | 145 | 1.07 | 7.35 | 130 | 0.90 | 12.2 |
| Giod | 1 | 198 | 3.18 | 16.13 | 198 | 1.00 | 6.2 |
| " | 2 | 118 | 1.11 | 9.41 | 98 | 0.83 | 8.8 |
| " | 3 | 210 | 1.13 | 5.41 | 74 | 0.35 | 6.5 |
| Lbz. | 1 | 295 | 4.65 | 15.84 | 360 | 1.22 | 7.7 |
| " | 2 | 75 | 1.31 | 17.52 | 130 | 1.73 | 9.9 |
| " | 3 | 62 | 0.805 | 12.97 | 78 | 1.25 | 9.7 |
| Sht. | 1 | 160 | 3.56 | 22.35 | 200 | 1.25 | 5.6 |
| " | 2 | 78 | 1.77 | 22.75 | 131 | 1.68 | 7.4 |
| " | 3 | 52 | 1.18 | 22.63 | 107 | 2.04 | 9.0 |
| Ats. | 1 | 220 | 3.53 | 16.06 | 288 | 1.31 | 8.8 |
| " | 2 | 180 | 2.10 | 11.73 | 204 | 1.13 | 9.6 |
| " | 3 | 212 | 1.62 | 7.70 | 142 | 0.67 | 8.7 |
| Bsso. | 1 | 250 | 4.30 | 17.24 | 312 | 1.25 | 7.2 |
| " | 2 | 128 | 1.50 | 11.69 | 166 | 1.30 | 11.1 |
| " | 3 | 212 | 1.74 | 8.19 | 182 | 0.86 | 10.5 |
| Kng. | 1 | 465 | 2.42 | 5.22 | 167 | 0.36 | 6.9 |
| " | 2 | 90 | 1.19 | 13.23 | 119 | 1.32 | 10.0 |
| " | 3 | 85 | 0.936 | 10.99 | 100 | 1.17 | 10.6 |
| Dck. | 1 | 325 | 2.08 | 6.41 | 166 | 0.51 | 8.0 |
| " | 2 | 62 | 0.536 | 8.65 | 60 | 0.97 | 11.2 |
| " | 3 | 40 | 0.468 | 11.7 | 50 | 1.24 | 10.6 |
| Lnn. | 1 | 228 | 2.22 | 9.72 | 116 | 0.51 | 5.2 |
| " | 2 | 68 | 0.93 | 13.67 | 141 | 1.41 | 10.3 |
| " | 3 | 78 | 0.96 | 12.31 | 100 | 1.28 | 10.4 |

tration test in a way that may be considered entirely new. The "concentration" feature is resident in the carbohydrate-nitrogen ratio. Our usual conception of "concentration" involves a correlation of measures of solute and solvent. We think of the amount of material dissolved in a unit of volume. There need not necessarily be such limitation to the concept "concentration." The idea may equally as well be referred to a correlation of the measures of two solutes. Thus, may we consider the amount of sugar excreted per unit of nitrogen and accept the nitrogen unit as the weight contained in one c.c. of urine. This is exactly what is done in estimating the carbohydrate-nitrogen ratio.

TABLE III

ANALYSIS OF SPECIMENS PASSED TWO HOURS AFTER BREAKFAST AND TWO HOURS AFTER LUNCH, DIETS UNCONTROLLED

| A.M. | 2 HRS. AFTER BREAKFAST SUGAR PER C.C. | | | A.M. CARBOHYDRATE RATIO % | | | P.M. | 2 HRS. AFTER LUNCH SUGAR PER C.C. | | P.M. CARBOHYDRATE RATIO % | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M. | G. | S. | M. | G. | S. | M. | G. | S. | M. | G. | S. |
| 10-19 | 0.54 | 0.92 | 0.69 | 4.5 | 7.5 | 9.5 | 1.02 | 0.65 | 2.00* | 7.0 | 8.9 | 14.7 |
| 10-20 | | 0.95 | 0.57 | | 7.1 | 7.1 | 0.82 | 0.68 | 0.75 | 7.0 | 7.7 | 8.1 |
| 10-21 | 1.02 | | 1.14 | 10.3 | | 10.0 | | | | | | |
| 10-23 | 1.42 | 1.80 | 0.59 | 6.7 | 7.2 | 6.7 | 1.64 | | 1.30 | 9.0 | | 10.8 |
| 10-24 | | 0.87 | 0.77 | | 5.7 | 13.9 | 1.16 | 0.61 | | 7.7 | 9.8 | |
| 10-25 | 0.75 | 0.80 | 0.45 | 4.8 | 8.8 | 9.6 | 0.76 | 0.38 | 0.58 | 6.4 | 7.6 | 9.3 |
| 10-26 | 1.16 | 0.90 | 0.98 | 8.9 | 10.3 | 11.5 | 1.41 | 0.57 | 1.41 | 9.2 | 10.3 | 12.1 |
| 10-27 | 0.79 | 1.02 | 0.75 | 7.0 | 9.4 | 10.2 | 1.18 | 0.54 | 1.05 | 8.2 | 7.7 | 15.0 |
| 10-28 | 1.15 | 1.24 | 1.22 | 9.1 | 9.8 | 13.4 | | | | | | |
| 10-30 | 1.27 | | 1.00 | 6.6 | | 12.4 | 1.18 | 0.72 | 1.54 | 7.7 | 10.2 | 10.7 |
| 10-31 | 1.00 | 1.01 | 0.82 | 7.8 | 7.7 | 13.2 | 2.53* | 0.73 | 1.18 | 17.3 | 8.2 | 11.4 |
| 11-1 | 1.10 | 1.39 | 0.60 | 8.7 | 10.3 | 9.5 | 1.67 | 0.63 | 1.49 | 12.5 | 8.9 | 11.1 |
| 11-2 | 1.17 | 1.16 | 0.91 | 6.9 | 9.5 | 11.2 | 1.64 | 0.86 | 1.32 | 10.4 | 8.0 | 13.1 |
| 11-3 | 1.29 | 1.06 | 0.77 | 6.8 | 8.9 | 15.7 | 1.38 | 0.86 | 1.34 | 8.6 | 8.0 | 14.3 |
| 11-4 | 2.13* | 1.48 | 0.80 | 17.2 | 9.5 | 12.1 | | | | | | |
| 11-6 | 1.33 | 1.25 | 0.76 | 7.1 | 9.5 | 8.8 | 1.42 | 0.56 | 0.76 | 9.1 | 8.4 | 8.7 |
| 11-8 | 1.31 | 1.21 | 0.59 | 9.5 | 8.6 | 8.0 | 1.27 | 0.90 | | 8.7 | 8.0 | |
| 11-9 | 1.21 | 0.98 | 0.53 | 10.5 | 7.8 | 7.7 | | 0.85 | 1.12 | | 8.6 | 10.5 |

| TOTAL NUMBER OF SAMPLES EXAMINED | | NUMBER OF SAMPLES WITH CARBOHYDRATE RATIO OVER 11.0% |
|---|---|---|
| G. | 30 | 0 |
| M. | 30 | 2 (both of these gave qualitative test for sugar) |
| S. | 31 | 15 |

*Qualitative Benedict's test for sugar present.

This method of expressing sugar concentration in urine has several distinct advantages over the older one of concentration in volume units. The excretion of sugar is independent of volume and a correlation of sugar and volume gives no useful information. Nitrogen is a renal excretory product, the rate of elimination of which is distinctly related to the time of intake of food. The curve for sugar excretion follows a quite similar path and is likewise related to the time of food ingestion. The nitrogen measure can admirably serve as a criterion by which to judge the significance of the concentration of other excretory products in a specimen of urine. To what extent observations of concentrations of sugar in urine, no matter how expressed, can favorably compare with the information fur-

nished by the measures of the total amounts excreted is to be determined only by the analysis of properly secured experimental data. In studying function in supposedly normal subjects that manner of expression of the measure of that function is the most useful which gives the most constant values. For under such conditions can we best select the group that gives minimal but significant deviations from the norm. From this point of view Tables I and II distinctly favor the carbohydrate-nitrogen ratio over the total amounts of sugar excreted as a measure of function. This is quite surprising to us, inasmuch as we are quite ignorant of any relationship between protein and carbohydrate metabolism that should account for such findings. Temporarily accepting this ratio as a measure of tolerance, a study of all our data based in largest part on the urinary findings after the test breakfast points to a carbohydrate-nitrogen ratio of 11 per cent as the upper limit of normal for a sample of urine passed 2 hours after the test.

TABLE IV

MIXED CARBOHYDRATE TEST MEAL

FOLLOWED IN TWO HOURS BY 30 GMS. DEXTROSE
SPECIMEN I PASSED AT 2 HOURS AFTER MEAL
SPECIMEN II PASSED AT 4 HOURS AFTER MEAL AND 2 HOURS AFTER DEXTROSE

| SPECIMEN I | VOL. C.C. | SUGAR PER C.C. | TOTAL SUGAR | C RATIO |
|---|---|---|---|---|
| | | | | N / % |
| G. | 70 | 1.25 | 88.0 | 8.9 |
| S. | 155 | 1.37 | 212.0 | 14.5 |
| M. | 108 | 1.15 | 124.6 | 12.4 |
| SPECIMEN II | | | | |
| G. | 56 | 1.56 | 87.0 | 9.0 |
| S. | 495 | 0.425 | 210.0 | 15.5 |
| M. | 114 | 0.96 | 110.0 | 11.0 |

TOTAL SUGAR FOR FOUR HOURS

| | |
|---|---|
| G. | 175 mgm. |
| S. | 422 mgm. |
| M. | 234 mgm. |

A ratio of higher percentage suggests intolerance, i.e., the concentration of sugar is greater than is to be explained by the concentration of nitrogen in the specimen.

The first attempt to test out this postulate gave us the data furnished in Tables III and IV. Three laboratory technicians, males under thirty and in good health collected their urines for the two-hour periods after breakfast and after lunch. Their diets were uncontrolled. Subject G. furnished 30 samples of urine in none of which the carbohydrate-nitrogen ratio was over 11 per cent. Subject M., 30 specimens, gave two samples that had ratios above 11 per cent in both of which the concentration of sugar was above 0.2 per cent and gave a distinctly positive qualitative test for sugar with Benedict's reagent. Subject S. in 31 samples gave 15 with a carbohydrate nitrogen ratio above 11 per cent in but one of which was the concentration of sugar as great as 0.2 per cent.

We fortunately happened upon three subjects in whom there appeared to be a distinct difference in reaction to their diets from the viewpoint of the

carbohydrate-nitrogen ratio. Subject G. stood out in marked contrast to subject S., the former giving no sample with a ratio above 11 per cent and the latter supplying half his samples with ratios above this figure.

All three subjects were then given the carbohydrate test breakfast reinforced, so to speak, by an added 30 gm. of glucose at the end of the first two hours for the purpose of clearly demonstrating a difference in tolerance, if such was a fact. The results are tabulated in Table IV. For the four hours following the meal subject G. excreted 175 mgm. carbohydrate, Subject M. 234 mgm., and Subjct S. 422 mgm. The carbohydrate-nitrogen ratio for the first and second specimens after the test were respectively for Subject G. 8.9 per cent and 9.0 per cent; for Subject M. 12.4 per cent and 11.0 per cent and for Subject S. 14.5 per cent and 15.5 per cent. In none of the samples of all three subjects was the concentration of sugar sufficiently large to give the ordinary qualitative test for sugar. After the same breakfast Subject S. passed 2½ times the amount of sugar that Subject G. did. The carbohydrate-nitrogen ratios for the corresponding samples after the test were much higher for Subject S. than for Subject G. This difference in their reactions to the test diet fits in perfectly with what we would have been called upon to prognosticate on the basis of their respective carbohydrate-nitrogen ratios in samples passed after uncontrolled diets. Considering that with three exceptions in 91 samples all the urines examined were sugar-free to the usual qualitative tests, and bearing in mind, likewise, that the carbohydrate-nitrogen ratio is an expression of concentration we are confronted with the proposition of presuming to detect sugar intolerance from urine samples "qualitatively free" of sugar. That this should be possible is not difficult to understand provided one considers the manner in which the factor of volume tends to nullify the value of the usual qualitative sugar test. A urine containing 0.4 per cent sugar gives a very decided qualitative test. If the same total amount of sugar is contained in 4 times the original volume, the sugar having 0.1 per cent concentration gives a negative qualitative test. But with dilution not only the sugar but all the urinary excretory products are diluted up in like measure. Under both circumstances the ratio of nitrogen to carbohydrate remains the same. The ratio must be as meaningful in one case as the other, as readily interpreted in the sugar "positive" sample as in the sugar-free one.

In diabetes mellitus a sugar-free (carbohydrate concentration of less than 0.2 per cent), specimen of urine must be accounted for by one of three conditions; (1) the diet contains no more carbohydrate than the subject will tolerate; (2) the specimen was excreted at a period so far removed from the last intake of food that the glycosuria resulting therefrom has disappeared; (3) the glycosuria following the diet has been masked by dilution. A method that would serve to distinguish condition one from conditions two and three would have the virtue of refining our diagnostic technic to the extent that sugar intolerance could be detected in urine samples qualitatively free of sugar. In the last analysis the process by which sugar dis-

appears from the urine under conditions two and three is one and the same. An excess of urinary sugar is contained in a volume sufficiently large to dilute the sugar to below a 0.2 per cent concentration. In the first instance (condition 2) the excreted sample being well removed from the time of the last food ingestion contains but minimal amounts of excess sugar and dilu-

TABLE V

TEST MEAL—MIXED CARBOHYDRATES

| SUBJECT | SPECIMEN NUMBER | VOL. URINE C.C | NITROGEN | | CARBOHYDRATE | | CARBOHYDRATE NITROGEN RATIO % | CLINICAL CONDITION; REMARKS |
|---|---|---|---|---|---|---|---|---|
| | | | TOTAL GM. | PER C.C MGM. | TOTAL MGM. | PER C.C. MGM. | | |
| P. | 1 | 210 | 3.48 | 16.59 | 313 | 1.49 | 9.02 | Mild diabetes |
| | 2 | 172 | 1.70 | 9.98 | 2310 | 12.35 | | |
| | 3 | 155 | 1.39 | 8.96 | 187 | 1.21 | 13.5 | |
| Dn. | 1 | 295 | 4.80 | 16.23 | 183 | 0.62 | 3.8 | Alimentary glycosuria |
| | 2 | 75 | 1.20 | 16.02 | 91 | 1.21 | 9.1 | Has had samples with 2 and 3% |
| | 3 | 95 | 1.32 | 13.95 | 121 | 1.27 | 10.3 | sugar |
| Pesk. | 1 | 148 | 3.55 | 24.01 | 370 | 2.5 | 10.3 | Mild diabetes |
| | 2 | 68 | 1.11 | 16.33 | 1045 | 16.1 | | |
| | 3 | 50 | 0.93 | 18.62 | 128 | 2.58 | 13.8 | |
| Pesk. | 1 | 178 | 2.85 | 16.02 | 274 | 1.54 | 9.6 | Same patient as above; test meal |
| | 2 | 58 | 0.94 | 16.3 | 178 | 3.07 | 18.9 | minus grape-nuts |
| | 3 | 36 | 0.53 | 14.78 | 49 | 1.36 | 9.2 | |
| Grgg. | 1 | 855 | 2.66 | 3.11 | 975 | 1.14 | 36.7 | Severe diabetes |
| | 2 | 118 | | | 2760 | 24.2 | | |
| | 3 | 120 | | | 3263 | 27.2 | | |
| Bonn. | 1 | 405 | 2.23 | 5.49 | 196 | 0.48 | 8.7 | Insurance applicants who had |
| | 2 | 60 | 0.65 | 10.90 | 94 | 1.57 | 14.3 | history of glycosuria |
| | 3 | 80 | 0.75 | 9.40 | 90 | 1.12 | 11.9 | |
| Brag. | 1 | 298 | 1.73 | 5.8 | 167 | 0.56 | 9.7 | Mild diabetes, blood sugar 4 |
| | 2 | 20 | 0.16 | 8.03 | 30 | 1.51 | 18.8 | hours after breakfast 0.216% |
| | 3 | 53 | 0.603 | 11.4 | 141 | 2.56 | 22.4 | |
| B.B. | 1 | 375 | 2.42 | 6.46 | 214 | 0.57 | 8.8 | Chronic seborrheic eczema |
| | 2 | 148 | 0.59 | 3.98 | 72 | 0.49 | 12.3 | |
| | 3 | 58 | 0.22 | 3.80 | 31 | 0.54 | 14.2 | |
| V.G. | 1 | 370 | 2.33 | 6.3 | 222 | 0.60 | 9.5 | Seborrheic eczema |
| | 2 | 58 | 0.61 | 10.43 | 94 | 1.62 | 15.5 | |
| | 3 | 100 | 0.90 | 9.03 | 132 | 1.32 | 14.6 | |
| Kgle. | 1 | 282 | 3.52 | 12.5 | 214 | 0.76 | 6.1 | Seborrheic eczema |
| | 2 | 150 | 1.48 | 9.87 | 185 | 1.23 | 12.5 | |
| | 3 | 50 | 0.51 | 10.08 | 89 | 1.78 | 17.6 | |

tion plays a minor rôle in the reduction of the sugar concentration below 0.2 per cent. In the second instance (condition 3) dilution plays the chief rôle in masking the glycosuria.

It has already been pointed out that the carbohydrate-nitrogen ratio is an expression of concentration that functions independent of volume and

for this reason might serve to distinguish between condition one and conditions two and three outlined above.

In Table V are listed some typical findings after feeding the test breakfast to subjects having a disturbed carbohydrate metabolism. In the study of this table attention should be paid to the absolute amounts of sugar excreted, the sugar concentration in the several specimens and the corresponding values of the carbohydrate-nitrogen ratio. Urines containing as much as 1 per cent sugar give carbohydrate-nitrogen ratios of 100 per cent or over and for such specimens no ratio value is listed.

A detailed analysis of the cases (Table V) discloses the following: Subject P. after passing over 2 gm. of sugar in the first two hours after the test breakfast excretes but 187 mgm. in the second two hours. This total quantity for the second two hours is well within normal limits; its concentration is but 0.13 per cent, a sugar-free sample. The corresponding carbohydrate-nitrogen ratio is 13.5 per cent and is the only evidence secured from this specimen that points to sugar intolerance.

Subject Dn with a true alimentary glycosuria, as proved after studies including blood sugar determinations, gives urinary findings that are within normal limits from all three aspects mentioned above.

Subject Pesk excretes over 1 gm. of sugar in the first two hours after the test; and but 128 mgm. in the second two hours. For this latter period the total amount of sugar excreted is within normal limits. The sugar concentration, however, is greater than 0.2 per cent and the sample gives a qualitative test for sugar. The carbohydrate-nitrogen ratio, too, is above the 11 per cent given as the upper normal limit. Only the concentration values in this sample reveal the sugar intolerance of this subject. This subject on half a test meal (meal minus grape-nuts) excretes for the four hours following the meal but 227 mgm. of carbohydrate with a positive qualitative test for sugar in only the first two hours' sample. In this same specimen the carbohydrate-nitrogen ratio is well above the allotted 11 per cent. Again the concentration figures bespeak an intolerance where the absolute measure of the excreted sugar fails to do so.

Subject G. is a severe diabetic excreting over 6 gm. of sugar in the four hours following the test breakfast. The control specimen (No. 1) though sugar-free gives the high carbohydrate-nitrogen ratio of 36.7 per cent. The total amount of sugar, 975 mgm., passed during this control period is three to four times as much as is voided by the other diabetic subjects listed in the table. It is quite clear that this subject even during the control period had not reovered from the effects of her last carbohydrate ingestion. This is strikingly borne out by the very high carbohydrate-nitrogen ratio and, in this instance, supported by the evidence of the total sugar excretion during this period.

Subject Bonn had a history of glycosuria. The only abnormality in his test meal is the carbohydrate-nitrogen ratio of 14.3 per cent in his first specimen after the test. Accepting this as evidence of intolerance does not permit including the subject in the group of diabetics or pre-diabetics except

insofar as the assumption is made that the excess sugar responsible for the increased ratio is glucose.

Subject Brag, a distinct though mild diabetic, excretes only 171 mgm. of carbohydrate in the 4 hours following the test breakfast, and only 30 mgm. in the first two hours. Still, the intolerance is clearly indicated in this sample by the carbohydrate-nitrogen ratio of 18.8 per cent.

The last three cases are included in the Table as illustrative of the clinical possibilities of a broadened scope of our interest in sugar metabolism. Seborrheic eczema has in many quarters been regarded as bound up with a disturbance of sugar metabolism though convincing laboratory evidence has not yet been presented. The findings in these 3 cases—we have records of but 4 in all—denote the presence of some sugar intolerance as judged by the carbohydrate-nitrogen ratios. If further study, by the method herein suggested or by others, is to give convincing evidence of the existence of disordered function founded on disturbances in carbohydrate metabolism apart from the sugars already known to the clinician, the identification of the chemical nature of the normal urinary sugars will be an all important consideration to medical science.

Strangely enough, after welcoming the technical procedures that promised to put into the discord the qualitative test for urinary sugar as a routine procedure for detecting intolerance, we are called upon to present evidence pointing to the superiority of the concentration test (carbohydrate-nitrogen ratio) over the measure of the absolute amounts excreted. We present the whole matter with a good deal of reluctance by reason of our failure to explain the physiologic mechanism behind our data. Just why the carbohydrate-nitrogen ratio values behave in a way to take on the significance they apparently do is not clear to us. Why the rate of excretion of nitrogen should act as if in a measure controlling the rate of excretion of carbohydrate cannot as yet be explained by any of the established facts in the physiology of nutrition. Yet the evidence is direct that in normal individuals following a standard diet, the measure of the excreted nitrogen is correlated with that of the excreted carbohydrate. That the values of these correlations should have greater constancy than the values of the absolute amounts of excreted carbohydrate after a test meal is equally puzzling. The manner in which this carbohydrate-nitrogen ratio has mirrored sugar intolerance from urine samples free of suspicion as judged by the usual standards compels further study of this relationship to the end that its true status be established.

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# THE KOTTMAN REACTION IN THE INSANE*

By A. M. Saunders, M.D.,† Chicago, Ill.

VARIATIONS in the function of the thyroid gland have been extensively studied in recent years. Many tests to estimate the degree of thyroid activity have come into the limelight. The basal metabolism test being most widely used at present, determines the amount of thyroid secretion by the rate of metabolism, while Goetsch's and Loewe's tests are based upon the peculiar sensitiveness to adrenalin in cases of excessive thyroid secretion. In view of the great importance of thyroid function upon bodily, as well as mental activities we wish to direct attention to a simple chemical test on blood serum, as devised by Kottman. In 1920 Kottman published his first report in the *Schweizer-Medizinische Wochenschrift* on a test to determine the amount of thyroid secretion by physicochemical means. In his extensive experimentations he used iodin and colloidal silver salts; the iodin on account of the particular relation it has to gland function and the silver because its photosensitiveness rendered it to be an excellent indicator. He found a variation in the blood sera of different thyroid conditions, which he attributed to a difference in the state of dispersion of the serum colloids. Dispersion is the incorporation of particles of one substance into the body of another. If the particles are fine a solution is formed, if somewhat coarser a gelatin, and if still more coarse an emulsion is produced. The size of colloid particles in blood and other body fluids is influenced by the amount of thyroid secretion. In excessive thyroid activity the particles are small, due to a protective element. In deficient thyroid secretion the particles are large.

In the Kottman test certain phenomena commonly observed in photography play a rôle. To make a photographic film a gelatin is mixed with silver iodide or silver bromide. The fresh mixture is in a highly dispersed state and is not very sensitive to light; it must first be permitted to ripen. During this ripening process the colloidal silver salt particles become larger and in that way become more photosensitive. In the Kottman test the blood serum is taking the part of the gelatin and a similar reaction might be anticipated. With greater photosensitivity of the silver iodide a presumably greater coarseness of the colloidal dispersion is present as is found in deficient thyroid secretion. If the photosensitivity is delayed the colloid particles have remained small, due to the excess of protective power of the blood serum.

Technic:

1 c.c. clear serum (Blood drawn before meals)
add 0.25 c.c. of 0.5 per cent K I
add 0.3 of 0.5 per cent of ag No. 3
Mix gently, expose five minutes to 500 Watt Mazda at distance of 25 cm.
Add 0.5 c.c. of 0.25 per cent solution of hydrochinone—Note color changes.

*Read at the Second Annual Meeting of the American Society of Clinical Pathologists, San Francisco, Calif., June 25, 1923.

†From the State Psychopathic Institute.

In carrying out this test a dark room is not absolutely necessary. A room with diffused daylight will not interfere with accurate results. The serum must be freshly drawn since old and contaminated specimens will interfere with the reaction. It should be clear and free from hemoglobin. The test tubes must be of even size and clean. A tube 1.5 by 8 cm. is satisfactory. The reagents must be freshly prepared and accurately measured. The color reaction after the addition of hydroquinon varies with different specimens. In a hyperthyroid case the original color persisted for a considerable time, while in normal specimens the color changed within about five minutes, while in sera from hypothyroid cases the color changed very rapidly. The blood serum from patients who have had bromides show a retardation, the bromides delaying the reaction as in ordinary photographic reactions. The temperature is important. We have kept the specimens as well as the reagents at ordinary room temperature for at least twenty minutes to insure uniformity of results.

The Kottman test is especially suitable in the diagnosis of thyroid disturbances in the insane. In those patients subjective symptoms are often misleading or indefinite, while objective symptoms are entirely lacking or distorted. The basal metabolism test is practically of no value in the mentally afflicted, since there is no cooperation nor understanding. This paper in a measure is a sequel to the report published by Peterson, G. Doubler, Levinson and Laibe in the journal of the American Medical Association in April, 1922. Out of 400 cases they have found a marked retardation of the reaction in 67 cases of clinical hyperthyroidism, while in most other diseases a normal reaction was obtained. In two patients suffering from an acute infection the reaction was accelerated, while in two cases of fright and extreme mental shock the reaction similar to hyperthyroidism was reported.

We have used the Kottman reaction on all incoming cases admitted to the Chicago State Hospital irrespective of any symptoms, age, sex, or classification. In Illinois every new patient must have the Wassermann test done, and we use part of the Wassermann blood serum as well as the spinal fluid for our investigation on the Kottman reaction. On 400 cases we used only the blood serum, while on 50 cases blood serum and spinal fluid were examined on the same patient.

The findings on blood and spinal fluid agreed in all of the 50 cases, except in 3 where blood was retarded, and spinal fluid normal. We found that about 21 per cent of all specimens of blood serum showed a retardation, while about 2 per cent revealed a very quick reaction. This latter 2 per cent of cases all showed upon subsequent examination some evidence of hypothyroid function.

Of the 105 cases giving a retardation a little less than half were frank hyperthyroid cases, with rapid pulse, excitability, and goiter. Most of the others had very indefinite symptoms. In 20 cases only a rapid pulse was found, with a slight difference in the pulse rate upon change of position from sitting to standing. In about 32 patients with a retardation of

the Kottman reaction no evidence of increased thyroid function could be obtained, several of them having a pulse rate of 60. These 32 cases were selected for further investigation. Eight of these were given thyroid extract feedings in increasing amounts for three days or less. One of those reacted promptly on the first day, with increased pulse and nervousness. Five showed a moderate reaction on the second day, with an increase in pulse rate and a difference of eight to twelve beats per minute on changing the position. Two of the eight did not react even after three days of thyroid administration. These were feeble-minded girls with physical evidence of disturbance of ductless glands functions.

On 24 patients with no evidence of thyroid hyperactivity we used subcutaneous injections of one to one thousand solution of adrenalin. All of these patients had marked positive reactions, as far as a local blanching of the skin, as well as the acceleration of pulse rate is concerned. Physical examination and past medical history on those patients revealed an unstable mental make-up, with periods of intense excitement.

We intend publishing a second report on this work with regard to the classification of mental diseases in cases of delayed Kottman reaction.

## CONCLUSIONS

The Kottman test is a simple test which can be rapidly carried out, and requires no elaborate equipment. If done accurately and interpreted in the proper way, it promises to give good index to thyroid activity. It is of great value in all kinds of patients, but decidedly superior to the basal metabolism test in the insane, and neurasthenics. It provides a method to estimate the frequency and extent of impaired function of the most important of ductless glands, the thyroid, in those that are becoming mentally unbalanced. Encouraged by our findings we wish to recommend this new and simple procedure for further investigation.

*(For discussion, see p. 425.)*

# THE ACCURACY AND CAPACITY OF THE PLATINUM LOOP*

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PROBABLY every one who has done routine bacteriologic testing of milk has regretted the time taken in the test for the total count with its multitude of pipettes and dilution bottles, all to be manipulated and then to be cleaned and sterilized for the next time. When it fell to my lot to do these tests, a few years ago for the City of Richmond, Va., it occurred to me that a simple loopful of milk plated out would at least distinguish between the good clean milks and the very dirty ones and that is really the information most desired. On trying it out I was surprised at the consistency with which the counts obtained by plating one loopful checked with the

Table I

Comparison of Plate Counts on Milk Samples Using Two Methods

| | Low counts | | | Medium counts | | | High counts | |
|---|---|---|---|---|---|---|---|---|
| | Loop method | Standard method | | Loop method | Standard method | | Loop method | Standard method |
| | 0 | 1 | | 13 | 21 | | 114 | 148 |
| | 1 | 0 | | 15 | 20 | | 130 | 115 |
| | 1 | 1 | | 18 | 29 | | 140 | 120 |
| | 2 | 0 | | 19 | 9* | | 140 | 132 |
| | 2 | 1 | | 19 | 31 | Total | 1524 | 515 |
| | 3 | 1 | | 20 | 6* | Ave. | 131 | 129 |
| | 3 | 1 | | 22 | 25 | Difference | 1.7% | |
| | 3 | 6 | | 35 | 46 | | | |
| | 4 | 3 | | 40 | 43 | | | |
| | 4 | 4 | | 42 | 44 | | | |
| | 5 | 4 | | 43 | 36 | | | |
| | 6 | 7 | Total | 286 | 310 | | | |
| | 6 | 11 | Ave. | 28.6 | 31.0 | | | |
| | 6 | 13 | Difference | 8.4% | | | | |
| | 8 | 9 | | | | | | |
| | 9 | 11 | | | | | | |
| | 12 | 9 | | | | | | |
| Total | 75 | 82 | | | | | | |
| Ave. | 4.4 | 4.8 | | | | | | |
| Difference | 9% | | | | | | | |

results obtained with the standard method. Table I shows the results of such a test made on a set of milk samples in the laboratory of the Bureau of Health, Richmond, Va. The figures are the actual counts and in the case of the standard method these should be multiplied by 1000; in the loop method by 900. This has not been done but the averages differ by approximately 10 per cent. The two instances in which there is most divergence in the results are marked with asterisks.

*Department of Bacteriology, Medical College of Virginia.
Received for publication, December 31, 1923.

So satisfactory were these results that it seemed desirable to determine more carefully just how accurate the ordinary platinum loop is as a measure in quantitative work. It has not been an easy matter to devise a method by which the volume of liquid held by a loop could be measured with accuracy. Bacteriologic plating methods are irregular and inaccurate. Direct weighing is not practicable, first because of the small size of the drop and second, because of the evaporation which causes constant change of weight.

Modern blood chemistry has developed very satisfactory methods for working with small quantities so some of these methods were tried. It was found that by adding 20 per cent of glucose to milk a distinct picramic acid color test could be produced with one loopful which could be compared in a colorimeter with known dilutions made with pipettes. However, consistent and accurate results were not obtained and the method was abandoned.

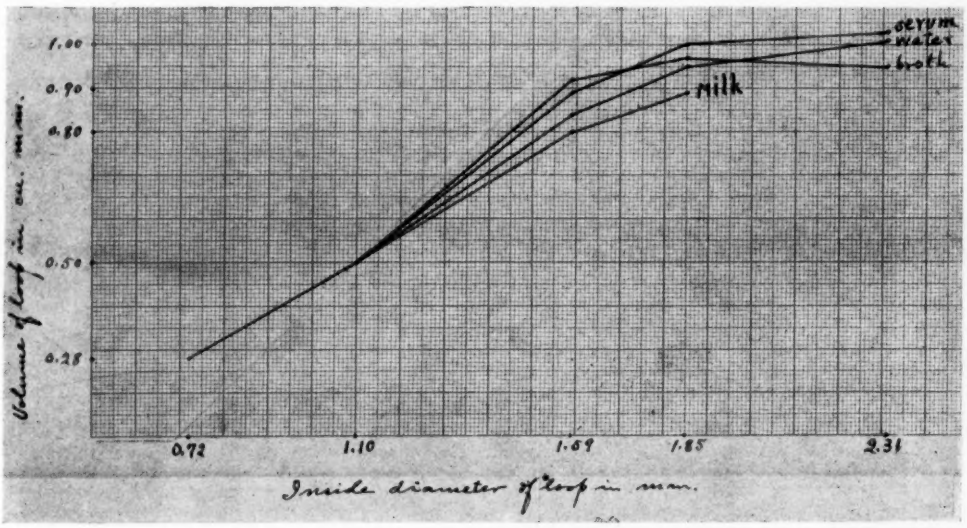


Fig. 1.

At last I hit on the following very simple method: the end of a fine glass capillary tube is plunged into the liquid in the loop, the liquid enters the capillary and then the capillary is applied to a scale and the length of the column of liquid measured. In making the test the loop is filled with the liquid, placed under a binocular microscope and then touched at the bottom edge with the end of the capillary. As soon as the liquid has entered the capillary the latter is applied to an accurate steel scale graduated in sixty-fourths of an inch.

Table II shows the results of such measurements on successive loopfuls using three different capillaries. Series I was made with a very fine capillary and gave a correspondingly long column of liquid; Series II was made with a larger capillary, and Series III with one of medium size. The results are quite uniform showing a deviation of less than 5 per cent from the mean. Some, at least, of these deviations are due to inaccuracies in the

test itself. For example, it could be seen under the microscope that some of the liquid was sometimes left on the wire of the loop and not taken into the capillary. Again, the capillary was emptied by blowing the liquid (water) out into filter paper, and then drawing air through it until the lumen remained open but no attempt was made to completely dry the inside of the capillary, nor is it probable that the same amount of water remained in the capillary each time. However, in spite of these recognized inaccuracies in the method of testing, the results are surprisingly uniform; so uniform in fact that many will be inclined to question their accuracy because we have all observed that loops sometimes take up a full round drop of fluid and sometimes only a thin film.

TABLE II

TESTS ON LOOP 4

Volume of Consecutive Loopfuls as Measured by Length of Capillary in 64ths of an Inch

| *Series I* | *Series II* | *Series III* |
|---|---|---|
| 160 | 52 | 80 |
| 152 | 51 | 80 |
| 153 | 50 | 82 |
| 158 | 52 | 80 |
| 159 | 52 | 83 |
| Average 156.4 | 52 | 76 |
| Maximum deviation, 4.4 or 2.8% | Average 51.5 | 77 |
| Average deviation, 1.5 or 1.0% | Maximum deviation, 1.5 or 2.9% | 80 |
| | Average deviation, 0.3 or 0.6% | 76 |
| | | 83 |
| | | 83 |
| | | 81 |
| | | 81 |
| | | 80 |
| | | 82 |
| | | 79 |
| | | 81 |
| | | 79 |
| | | 79 |
| | | 79 |
| | | 78 |
| | | 79 |
| | | 76 |
| | | Average 79.7 |
| | | Maximum deviation, 3.7 or 4.6% |
| | | Average deviation, 0.8 or 1.0% |

Obviously, to obtain such uniform results the loop has been used in this work according to a definite technic which is as follows:

1. The loop should be well made, round and smoothly soldered at the junction point, or even better, electrically welded.

2. The loop should be held with the plane of the loop in a vertical position and it should be submerged in the liquid only enough to fully cover the loop.

3. The loop should be withdrawn from the fluid slowly. As the loop comes up out of the fluid the fluid drains downward under the combined influence of gravity, surface tension and viscosity and tends to assume a

definite biconcave shape. The loop should be withdrawn so slowly that these forces are at all times in equilibrium and especially at the end when the pulled-up portion of the surface which adheres to the loop finally snaps off and the loop is free. This slow removal of the loop from the surface of the liquid is essential if uniform results are to be obtained.

These tests show that successive loopfuls are very uniform in size but have not shown just what that size is in any of our well-known units. This is, however, very easily determined by weighing. The single loopful cannot be weighed accurately but ten or twenty can be weighed with an accuracy quite sufficient for our purpose. To do this, one cubic centimeter of fluid is placed in a homeopathic vial, corked and weighed. Then the required number of loopfuls are removed and the vial again weighed. The difference in weight divided by the number of loopfuls removed, gives the weight of each loopful. If distilled water is the fluid being used the loopfuls are removed from the wire simply by placing the latter in the flame. If the fluid contains inorganic salts which would accumulate on the wire such fluid should be first removed from the loop by washing, before the latter is placed in the flame.

TABLE III

EFFECT OF SURFACE TENSION ON SIZE OF LOOPFUL

| SOLUTION | | DYNES PER SQ. CM. | WEIGHT OF ONE LOOPFUL |
|---|---|---|---|
| NaOH sol. | 1-64 | 62 | 0.99 |
| " | 1-4 | 55 | 1.00 |
| " | 1-1.75 | 44 | 0.98 |
| " | 1-1.25 | 37 | 0.98 |
| " | 1-1 | 36 | 1.00 |
| Soap sol. | 1-64 | 51 | 0.85 |
| " | 1-32 | 43 | 0.83 |
| " | 1-16 | 39 | 0.80 |
| " | 1-1 | 25 | 0.72 |

Fig. 1 shows the results of such tests on loops of several sizes and four different fluids. Note (1) that the abscissae represent the diameters of the loops, the ordinates the volumes held by the loops. These diameters are the inside diameters as measured by an ocular micrometer and a thirty-two millimeter objective. If a loop varies somewhat from round the average diameter is taken. (2) Six loops were used having diameters of 0.72, 1.10, 1.59, 1.85, 2.31 and 3.00 millimeters. The three millimeter loop could not be used in this method as the film was apt to break. All the loops were made from number twenty-six platinum wire. (3) With the smaller loops the volume held by the loop increases with the diameter, though a little less rapidly. (4) With the larger sizes the amount held is nearly constant, irrespective of the diameter of the loop and is not far from one cubic millimeter. (5) There is some difference in the amount of the different fluids held by the larger loops. This difference however is not large, loop 3 holding betwen 0.8 and 0.9, loop 4 between 0.9 and 1.0 and loop 5 between 9.5 and 1.05 cubic millimeters.

The exact reason for this difference is not known. One naturally thinks of surface tension as the probable cause of these differences in volume. To test this out soap solutions of various strength were made up to give a range of surface tension and then the size of the loopfuls were measured. Table III shows at the bottom the results obtained with the soap solutions, which seem to indicate that some change of volume of fluid follows change in surface tension. However, these soap solutions gave some peculiar and inconsistent results which I do not understand and so I have very little confidence in these results. I suspect that the colloid state of these fluids affects the size of the loopfuls but I have no data on this point.

To get away entirely from colloid solutions I made up a series of dilutions of sodium hydroxide in water which gave a range of surface tension. On measuring the size of loopfuls from these solutions you will note that there was no variation. The results were constant and were not affected by variation in surface tension, so it seems that surface tension *per se* does not affect the volume held by these loops. Viscosity also should not affect the volume of the loopful, provided the loop is withdrawn from the liquid so slowly that the viscous fluid has time to reach equilibrium.

When we stop to consider it seems remarkable how dependent we have been on pipettes for measuring small quantities of fluids accurately. Even so, we have not been able to measure less than one-tenth of a cubic centimeter with any great accuracy. It looks now as though the common platinum loop can be used as a fairly accurate measure of quantities of a still smaller order, and thus in some cases save the trouble of the usual dilution methods.

# LABORATORY DIAGNOSIS IN THROAT INFECTIONS*

## I. The Blood Plate as an Aid to Diagnosis

By C. C. Young and Minna Crooks, Lansing, Michigan

THE routine use of the blood plate for the diagnosis of throat infections was adopted in the laboratories of the Michigan Department of Health in November, 1919. This was done to meet the demand for a laboratory diagnosis in septic sore throat, and to serve as a control on diphtheria diagnosis. It has proved to be a valuable aid to laboratory diagnosis and a practical routine procedure. Swabs submitted for dignosis are streaked, first, on a blood plate; second, on Loeffler's serum medium; and third, smeared on a slide for immediate examination. The streptococcic findings were routinely reported beginning January, 1920, after a careful study had been made of the significance of the blood plate findings and the value of the bacteriologic results to physicians and health officers.

Table I

Throat Infections

| | Diphtheria | Septic Sore Throat | Vincent's Angina |
|---|---|---|---|
| Causative Agent: | B. diphtheriae | Streptococcus hemolyticus (Beta type, Brown 1919) | Spirocheta and B. Fusiformis |
| Symptoms: | Temp. 99-103<br>Membrane present | Temp. 101-105<br>Pseudomembranes (Park and Williams) | Temp. none<br>Membrane present |
| Lab. Diagnosis: | Loeffler's serum media 8 to 18 hrs. incubation.<br>Direct examination. | Blood agar 18 to 24 hrs. incubation. | Direct examination |
| Therapeusis: | Antitoxin. | Rest in bed. | Neoarsphenamine (Morgan, 1923) |
| Prophylaxis: | Quarantine, antitoxin and toxin-antitoxin. | Isolation and gargle. | Gargle. |
| | **Bacteriology** | | |
| | B. diphtheriae | Hemolytic streptococci | |
| Distribution: | Etiologic organism in diphtheria. May be found in wounds, all mucous surfaces, normal throats, milk, feces and fomites. | Etiologic organism in septic sore throat, pneumonia, empyema, septicemia, erysipelas, impetigo contagiosa, etc. Secondary invader in scarlet fever, measles, influenza. May be found in normal throats, excised tonsils, milk, feces and fomites. | |
| Pathogenicity: | Death by toxemia. | Death by septicemia. | |
| Virulence Test: | Fatal for 250 gm. guinea pig in 1 to 3 days.<br>Virulence constant. | Fatal for mice in 12 to 48 hrs. Virulence lost in culture. (NaKayauea, 1920). | |
| Transmission: | Contact, carriers and fomites. | Contacts, carriers and fomites. | |

*From the Bureau of Laboratories, Michigan Department of Health, Lansing, Michigan.
Received for publication, October 9, 1923.

An analysis of throat infections as set forth in Table I showed that septic sore throat had the same place in laboratory diagnosis as diphtheria, i.e., confirmation of the physician's diagnosis and control of quarantine.

### PATHOGENICITY OF STREPTOCOCCI

Doctor F. F. Russell, in a discussion on hemolytic streptococcus carriers, at a meeting of the Laryngological Society in 1919, referred to epidemics of streptococcus infection as being among the oldest problems with which the

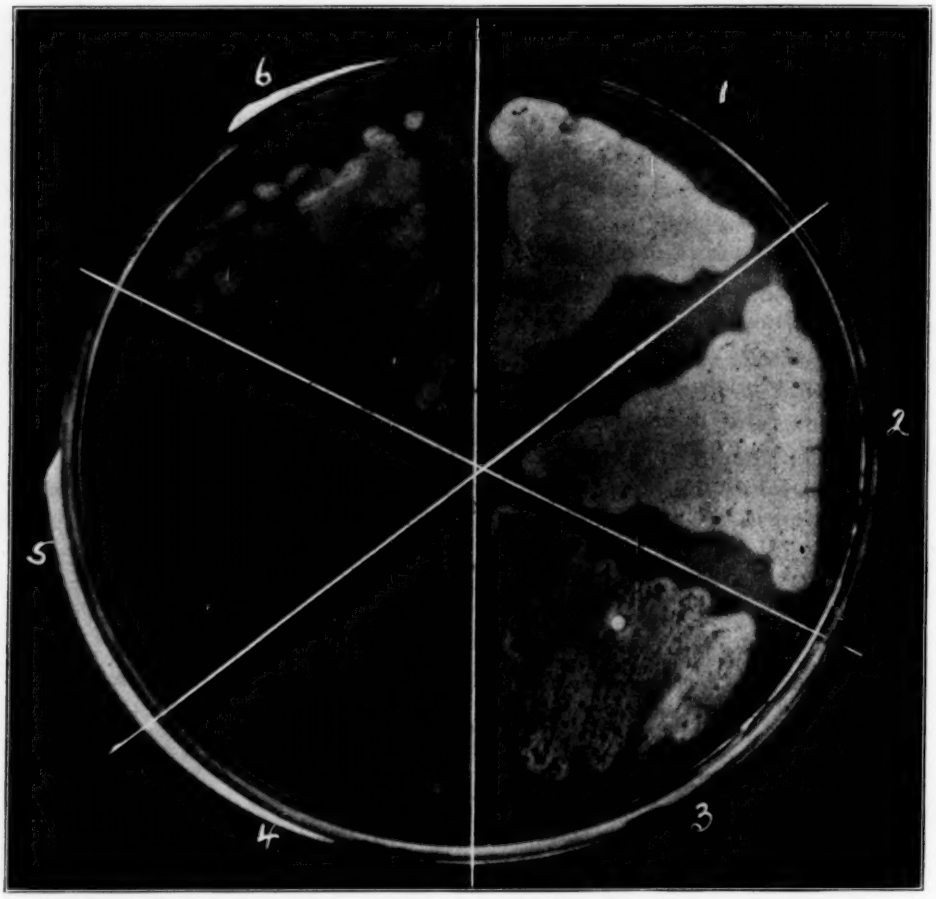


Fig. 1.—Photograph of routine blood plate. Inoculated December 21, 1921. Reported December 22, 1921. Medium: Liebig's extract, Difco peptone agar and sheep's blood 5 per cent. Segments 1 and 2: 90 to 95 per cent, Type IIIa Betahemolytic streptococci (characteristic blood plate picture of septic sore throat). Segment 3: Type IIIa and B.-diphtheria. Plate from mixed infection. Segments 4 and 5: Green-producing streptococci. Segment 6: Type IIIb streptococci (characteristic culture from convalescent septic sore throat).

medical profession has had to deal. The medical records of the Base Hospitals, made during the winter of 1917-1918, and the reports subsequently published from the various cantonments, show all too convincingly the pathogenicity of streptococci in the deaths and disabilities of our soldiers.

The survey of the literature points to Beta hemolytic streptococcus as the etiologic organism in septic sore throat (Smillie, 1917) and indicates a

greater pathogenicity of streptococci with production of hemolysis (Clawson, 1920) than of streptococci with production of methemoglobin.

## BLOOD PLATE READINGS

Before any diagnostic value can be attached to blood plate readings, the preparation of the blood plate and the technic of inoculation must be taken into consideration. The results obtained in the army were by the procedure recommended by the commission on standard routine methods. This procedure was a surface inoculation by the streak method. The surface inoculation and the poured plate method, as described by J. H. Brown (1919), ought to give comparable results and show the proportion of organisms present for which blood agar is selective. The enrichment method as used by others would indicate the presence of any one or more organisms, without giving their relation in numbers to the throat flora.

The blood plate, as adopted in the laboratories of the Michigan Department of Health, consists of two per cent beef extract agar adjusted to pH 7.0 to which five per cent sheep's blood and five-tenths per cent sodium chloride have been added. Plates are poured daily and incubated over night before using. As shown in Fig. 1, one plate is divided into six segments and used for the inoculation of six swabs. After 18 to 24 hours' incubation at a temperature of 37° C., the plates are read. The colonies are identified by a macroscopic examination and confirmed by smears for morphology and reaction to Gram's method of staining. Based upon our observation that an invading organism wipes out the normal flora, only those cultures showing 90 to 100 per cent of the growth are considered diagnostic. The bacteriologist notes the percentage of the streptococcus colonies on the plate cultures and classifies them by the following key:

### STREPTOCOCCI

Gram positive cocci arranged in pairs or chains growing well only on enriched media.

Key to the identification on blood plates.

| | |
|---|---|
| *A. Methemoglobin produced.* | *Green-producing streptococci* |
| B. Methemoglobin produced with hemolysis. (Alpha type, Brown's classification) | Type I. |
| BB. Methemoglobin produced without hemolysis. | |
| C. Bile Soluble. | *Pneumococci* |
| D. Agglutinated in antipneumococcus sera. (Avery, 1918) | Types I, II & III. |
| DD. Not agglutinated in antipneumococcus sera. | Type IV. |
| *AA. Hemolysis produced.* | *Streptococcus hemolyticus* |
| B. Colonies surrounded by a narrow zone of hemolysis. | Type II. |
| C. Minute punctate colony. | Type IIa. |
| CC. Large colony 1 to 2 mm. in diameter. | Type IIc. |
| BB. Colonies surrounded by a wide zone of hemolysis. (Beta hemolytic streptococci, Brown's classification) | Type III. |
| C. Minute punctate colony. | *Type IIIa. |
| CC. Large colony. | Type IIIc. |

*The hemolytic streptococcus that we designate as "Type IIIa" has a definite etiologic association. Our bacteriologic findings for the past two years have pointed to this organism as the principal causative agent in serious secondary infections. Other types of streptococci may be the cause of pathologic conditions, however.

### DISTRIBUTION

After the standardization of the blood plate, the next problem that presented itself was the determination of the relation of the incidence of hemolytic streptococci to disease, for their wide distribution would seem to indicate the presence of the streptococcus to be of little diagnostic value. Our studies and analysis of the investigations of others show us that Beta hemolytic streptococci can be recovered from normal throats, hypertrophied tonsils, milk and fomites. The same is true of one of our best known and best controlled pathogens, i.e., B. diphtheriae. The incidence of hemolytic streptococci is much higher than diphtheria bacilli in throats without acute inflammation; nevertheless, we believe the finding of hemolytic streptococci in normal throats has the same relation to diagnosis in throat infections as the finding of diphtheria-like bacilli in normal throats. This problem is not one of diagnosis, but the identification of carriers. The high incidence of hemolytic streptococci reported in tonsils and in normal throats led us to study the bacteriology of excised tonsils and throat swabbings in relation to the pathology in order to determine the diagnostic significance of streptococcal findings.

### SIGNIFICANCE OF BACTERIOLOGIC FINDINGS

It is our conclusion that there is a definite relation between the amount of growth and the pathology of tissues, and that when hemolytic streptococci are recovered in practically pure culture they have a diagnostic significance. This has been confirmed by others (Otteraaen, 1920, Davis, 1921, Kraft, 1921, Pilot, 1921).

In 1920, Otteraaen examined the throats of 300 patients for hemolytic streptococcus. These patients were ill with acute infectious diseases. He used both the army medical department method and the enrichment method, and claims that there was general distribution of hemolytic streptococcus in both normal individuals and persons ill with acute infections. He did not, however, report hemolytic streptococci in practically pure culture by either method, but reports the incidence by the enrichment method to be much higher.

Davis, in 1921, examined three groups of students, consisting of fifteen each, to determine the occurrence of hemolytic streptococci in normal throats. He used the poured plate method, and found that hemolytic streptococci occurred in all throats at least once during the two months of observation. He noted the number of streptococci in relation to other organisms, and found they comprised 1 to 10 per cent, with one very interesting exception, i.e., the culture from a young man who was feeling quite normal when the swab was taken and showed no abnormal condition of the throat, yielded an abundant, almost pure culture of hemolytic streptococci. In twenty-four hours, he was ill with typical septic sore throat.

Doctor Adolph Kraft of the Department of Pathology, University of Illinois, in a report on hemolytic streptococci as an etiologic factor in suppurating appendices, says, "When hemolytic streptococci occur in pathogenic

appendices, they are present in large numbers and in practically pure culture, while when found in normal appendixes they are few in number."

Pilot and others of the University of Illinois, in 1921, found no relation between the streptococcus findings of the surface swabbings and the cultures from the inside of the tonsils. Doctor Pilot made his study on 103 children with no evidence of any recent inflammation, fever, or subjective symptoms of sore throat. He found that in both the throat swabs before tonsillectomy and the surface cultures of the tonsils, hemolytic streptococci were relatively few in number, while the cultures from inside of tonsils showed comparatively a high per cent of growth.

Doctor Ramsey, (1922) in a report on throat infections in the Boys' Industrial School, states that "Cultures taken from the interior of the tonsils showed higher percentage of hemolytic streptococci than swabs taken before operation."

Our studies made during 1922 and 1923, on 850 tonsillectomies showed similar results. Specimens were submitted to us by the Bureau of Institutional Health Administration of the Michigan Department of Health from the state penal and correctional institutions. Throat swabs were taken from both of the tonsils and from the nasopharynx of each case before operation. The specimens were delivered to us by messenger, thus avoiding all delays in transit. Swabs were cultured by the streak method upon routine blood plates, and material for culture was collected from the inside of the tonsil, after searing and cross-sectioning to avoid contamination. The tonsils were hypertrophied and many of them contained pus, but the throats were without acute inflammation. A high percentage of the tonsils showed hemolytic streptococci in practically pure culture, while the throat and nasopharynx swabs showed staphylococci or mixed cultures in which hemolytic streptococci comprised only a small percentage of the growth. We found no relationship between the bacteriology of hypertrophied tonsils and throat flora.

Throat swabs submitted routinely from the admissions to the state penal institutions furnished the material for the comparison of the flora of normal and diseased tissue in throats. Over 5000 swabs were examined during 1921 and 1922 from throats showing no pathology and from the throats of individuals ill with infectious diseases. Hemolytic streptococci were routinely recovered from throats without pathology but comprised only a small percentage of the growth, except in the Boys' Industrial School in which a survey was made previous to a scarlet fever epidemic, and in one of the reformatories during an epidemic of septic sore throat. In both instances, practically pure cultures of hemolytic streptococci were recovered from normal throats as well as from infected throats.

## THE VALUE OF THE LABORATORY FINDINGS TO CLINICIANS

Two distinct advantages result from the use of the blood plate: (a) a negative diphtheria finding from a sore throat simulating diphtheria does not mean a missed laboratory diagnosis, and (b) a positive hemolytic streptococcus finding makes possible the state regulation requiring the isolation

of septic sore throat. The economic value of differentiating between septic sore throat and diphtheria is obvious, for one involves quarantine and the other isolation. During the four year period previous to 1919, only 22.9 per cent of the swabs submitted for diagnosis gave positive results in this laboratory. This finding is comparable with the results of other state laboratories. Our results in the laboratory during 1919 showed about the same proportion of positive and negative findings. Of 2486 throat swabs submitted by clinicians from cases of sore throat we found diphtheria bacilli on 698 swabs. In other words we were unable to account bacteriologically for 1788 cases of sore throat which were potentially diphtheria, and in many instances, as manifested by the complaints of the clinicians sending in the swabs, we had submitted negative findings on cases of "typical diphtheria." In 1921, of the 9448 swabs submitted for diagnosis, 1864 showed diphtheria, 3044 hemolytic streptococci, 2305 green producing streptococci, and 114 the organisms of Vincent. Consequently we had reduced our negative findings to a little more than 21 per cent.

There is further evidence of the value of streptococcus findings in Table II which shows the tabulated results of our routine examinations on throat swabs in 1921.

TABLE II

THE RELATIONSHIP OF BACTERIOLOGICAL FINDINGS TO CLINICAL HISTORIES—1921

| CLINICAL DIAGNOSIS | LABORATORY FINDINGS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Direct Smear | | Serum Media | | Blood Plate | | |
| | | K. L. | Vin. | B. diphtheria | IIIa | H. S. | G.S. | Negative |
| Diphtheria | | | | | | | | |
| Septic Sore Throat | 482 | 30 | 8 | 36 | 178 | 88 | 78 | 34 |
| Vincent's Angina | 9 | | 3 | 4 | 2 | | | |
| Tonsillitis | 560 | 22 | 1 | 88 | 195 | 113 | 95 | 46 |
| Doubtful | 242 | 8 | 1 | 52 | 40 | 90 | 9 | 42 |
| | 2040 | 263 | 21 | 457 | 545 | 337 | 229 | 159 |

K. L. = Klebs-Loeffler bacilli.
Vin. = Organisms of Vincent.
H. S. = Hemolytic streptococci.
G. S. = Green-producing streptococci.
IIIa = Type IIIa, hemolytic streptococci.

Of the 2040 swabs submitted with complete information, giving a history of fever or membrane, or both, 747 were diagnosed diphtheria, 1051 were designated clinically as septic sore throat, and in 242 cases the doctor was clinically unable to differentiate between septic sore throat and diphtheria. Table II shows the distribution of bacteriologic findings compared with clinical diagnosis. There is only relative agreement between the bacteriology and clinical diagnosis. For example, only 480 positive diphtheria findings were reported on 747 diphtheria diagnoses, and 178 streptococcus findings reported on 482 septic sore throat diagnoses. Conversely, 176 Beta hemolytic streptococcus findings were reported on 474 diphtheria diagnoses, and 66 positive diphtheria findings on 482 septic sore throat diagnoses.

SUMMARY

The determination of the etiologic organism in throat infections is of value in differential diagnosis as a prerequisite in serum prophylaxis and in quarantine. The bacteriologic technic should be as simple and rapid as possible and should comprise a method requiring not longer than twenty-four hours. We have shown a standard blood plate to be a very satisfactory means of determining the etiologic organism in septic sore throat, thereby differentiating pseudomembranous throat infections from diphtheria. We have, by the report of our studies and by referring to the literature on the investigations of others, brought evidence to show that the wide distribution of hemolytic streptococci does not mask the diagnostic value of the finding of streptococci in practically pure culture. We have also shown that a pure culture of hemolytic streptococcus is indicative of pathogenicity. We have shown an increase from 2486 swabs for diagnosis in 1919 to 9448 in two years, of which 63 per cent, as indicated in Table II, were from throat infections other than diphtheria.

COMMENT

The blood plate is of particular value in determining for the clinician cases showing streptococci of known pathogenicity, thereby giving him the prerequisite in isolation, and establishing septic sore throat as a disease entity. It is, therefore, recommended as an aid to laboratory diagnosis in throat infections.

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# *TRANSACTIONS*

## PROCEEDINGS OF THE SECOND ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS, SAN FRANCISCO, CALIF., JUNE 25, 26, 1923

THE meetings were held in the Municipal Auditorium Building in a room reserved for the Society through the kindness of the local arrangement committee of the American Medical Association.

### First Session—June 25, 1923

Meeting called to order at 9.00 A.M. by President Hillkowitz. The minutes of the First Annual Meeting held at St. Louis were read by Secretary Burdick who also submitted the following report as Treasurer:

| | |
|---|---|
| Income from Dues | $1852.10 |
| Expenditures | 915.19 |
| Balance on hand June 1, 1923 | 936.91 |

On motion both minutes and report were approved.

The President appointed a nominating committee consisting of Drs. Ernst A. Victors, Willis P. Butler and Michael G. Wohl.

The advisability of a uniform terminology in the diagnosis of pathologic specimens and particularly of neoplasms was the first subject taken up for discussion. Dr. Wm. C. McCarty, of Rochester, Minn., after presenting his views on the present status of the problem moved that a committee be appointed to study the matter and report at the next meeting. The motion was carried.

The following report of the Committee on standardization of Laboratory Reagents was then presented to the Society:

#### Report of Committee on Standardization of Laboratory Reagents

The attached report represents the attempt of your committee to set tentative standards upon those biological reagents which are in common use in diagnostic laboratories. The final report represents the consensus of opinion of the members of the committee.

Several interesting points have arisen which the committee feel should be decided by the association as a whole. One member of the committee desires that the scope of the standards be widened to include the methods of testing, as well as the reagents. Another member desires that proteins, pollens, etc., be also included. It was not felt wise at this time to attempt this, without specific instructions from the society. It will be noted that in one instance, hemolytic serum against sheep corpuscles, that one member feels that the standard is somewhat too high, that serum testing in a dilution of 1:5000 would answer the requirements.

It should be realized that the standards set are tentative, and represent the usual practice at this time. The committee welcome any suggestions or criticisms that will help in this mattter.

Respectfully submitted,

F. M. Huntoon, Chairman.
J. H. Black,
J. A. Kolmer,
A. H. Sanford,
R. G. Stillman.

**Report of the Committee on the Standardization of Laboratory Reagents.—F. M. Huntoon, Chairman.**

## I. Standardization of Antigens for Agglutination

1. *By using an Antigen as standard.*

The standard agglutinating antigen should be prepared according to the method outlined by the Department of Pathology, University of Oxford. The standard antigen may be used for a criterion for six months.

*Preparation:*

The bacillus is grown for 24 hours at 37° C. in ordinary veal peptone bouillon (P. H. depending upon the organism) in large Erlenmeyer flasks partly filled (1 liter of bouillon in a one and a half liter flask).

Before use, the flasks of bouillon are sterilized in the autoclave at 115° C. for not more than 15 minutes, and are then tested for sterility by incubation at 37° C. for 48 to 72 hours.

They are inoculated with a few drops each from a 20 to 24 hours old bouillon culture of the bacillus (B. typhosus, B. paratyphosus, etc.).

The culture used should be one which has been subcultivated daily in bouillon for one or two weeks (or longer). This continued subcultivation has the effect of increasing its agglutinability and diminishing any tendency to spontaneous agglutination.

At the end of 20 to 24 hours' growth at 37° C., the flasks are well shaken and to each is added 0.1 per cent (1 c.c. per liter) of commercial (40 per cent) formalin. They are again shaken and placed in a cold chamber in the dark at about 2° C.

At intervals on the same day, and on subsequent days for 4 or 5 days, the flasks are again thoroughly shaken and replaced at once in the cold chamber.

After 3 or 4 days, they will be found to be absolutely sterilized. Should it happen that the bacterial suspension is not entirely homogeneous, it may be shaken for some hours in a mechanical shaker, or may finally be filtered through sterile cotton wool.

New antigens to be tested should be diluted to an opacity equal to that of the Standard Antigen. The two antigens are then tested simultaneously against a serum of known strength. The new agglutinating antigen should agglutinate in the same dilution as the Standard Antigen.

*Alternative method for preparation of standard antigens.*

With organisms not giving good growths in broth media, growth must be secured on suitable solid media, removed by washing with salt solution and finally treated as in the standard method.

Suggestions for each antigen are invited from the members of the society.

2. *By using a serum as standard.*

The Standard Serum is prepared by injecting rabbits with the organism used in the antigen. The serum is dried on paper and the unit determined by a titration with an antigen prepared in the same way as the Standard Antigen. The paper can be preserved by sealing in a vacuum and placing in the cold storage. It can be used for six months as a Standard Serum.

Freshly prepared serum must show the same number of agglutinin units as the Standard Agglutinating Serum in comparative tests with any antigen.

*Specificity.*

Antigens for agglutination must be tested for specificity with Standard Serums prepared from closely allied organisms known to give group reactions.

## II. Standardization of Serums for Agglutination

1. *By using a Standard Agglutinating Antigen as Standard Potency*

Each serum must show as many agglutinin units as is set as its individual standard of potency when tested against the Standard Agglutinating Antigen.*

*See Section I-1.

*Specificity*

Each serum must be tested against Standard Agglutinating Antigens prepared from all closely related types of organisms known to give group reactions. Its specificity must conform to the individual standards.

Antigen, B. abortus (Equine) for agglutination test
Antigen, B. abortus (Bovine) Bang, for agglutination tests
Antigen, B. anthrax (for agglutination)
Antigen, V. cholera
Antigen, B. coli Polyvalent
Antigen, B. coli communior
Antigen, B. coli commnis
Antigen, B. enteritidis
Antigen, B. dysentery Shiga, for agglutination
Antigen, B. dysentery Flexner, for agglutination
Antigen, B. dysentery Hiss, for agglutination
Antigen, B. dysentery Strong, for agglutination
Antigen, B. mallei, for agglutinaiton tests
Antigen, Meningococcus, Type I
Antigen, Meningococcus, Type II
Antigen, Meningococcus, Type III
Antigen, Meningococcus, Type IV
Antigen, M. melitensis
Antigen, B. pertussis
Antigen, B. pestis
Antigen, B. plague
Antigen, B. typhosus
Antigen, B. paratyphosus A
Antigen, B. paratyphosus B (for agglutination)
Antigen, B. typhosus (for agglutination)
Antigen, B. paratyphosus A (for agglutination)
Antigen, B. paratyphosus B

| | MINIMUM SPECIFIC TITRE | MAXIMUM GROUP AGGLUTINATION | | INCUBATION TIME | INCUBATION TEMP. |
|---|---|---|---|---|---|
| Serum Agglutinating, B. abortus Bang | 1:800 | | | 4 hr. | 37.5° C. |
| Serum Agglutinating, V. cholera | 1:1600 | | | 2 hr. | 37.5° C. |
| Serum Agglutinating, B. coli communis | 1:800 | Communior | 1:300 | 2 hr.<br>18 hr. | 37.5° C.<br>Cold Stg. |
| Serum Agglutinating, B. coli communior | 1:800 | Communis | 1:300 | 2 hr.<br>18 hr. | 37.5° C.<br>Cold Stg. |
| Serum Agglutinating, B. coli Polyvalent | 1:1600 | Communis<br>Communior<br>Aerogenes | | 2 hr.<br>18 hr. | 37.5° C.<br>Cold Stg. |
| Serum Agglutinating, B. dysentery Shiga | 1:800 | Flexner<br>Hiss<br>Strong | 1:50<br>1:50<br>1:50 | 2 hr.<br>18 hr. | 37.5° C.<br>Cold Stg. |
| Serum Agglutinating, B. dysentery Flexner | 1:1600 | Hiss<br>Strong | 1:300<br>1:300 | 2 hr.<br>18 hr. | 37.5° C.<br>Cold Stg. |
| Serum Agglutinating, B. dysentery Hiss | 1:800 | Flexner | 1:300 | 2 hr. | 37.5° C. |
| Serum Agglutinating, B. dysentery Polyvalent | 1:1600 | Shiga<br>Flexner<br>Hiss<br>Strong | | 2 hr.<br>18 hr. | 37.5° C.<br>Cold Stg. |
| Serum Agglutinating, B. enteritidis | 1:800 | Any Dysentery | 1:100 | 2 hr.<br>18 hr. | 37.5° C.<br>Cold Stg. |

| | MINIMUM SPECIFIC TITRE | MAXIMUM GROUP AGGLUTINATION | | INCUBATION TIME | INCUBATION TEMP. |
|---|---|---|---|---|---|
| Serum Agglutinating, Meningococcus Type I | 1:400 | Ty. II<br>Ty. III<br>Ty. IV | 1:25<br>1:50<br>1:25 | 18 hr. | 45° C. |
| Serum Agglutinating, Meningococcus Type II | 1:400 | Ty. I<br>Ty. III<br>Ty. IV | 1:25<br>1:25<br>1:50 | 18 hr. | 45° C. |
| Serum Agglutinating, Meningococcus Type III | 1:400 | Ty. I<br>Ty. II<br>Ty. IV | 1:50<br>1:25<br>1:25 | 18 hr. | 45° C. |
| Serum Agglutinating, Meningococcus Type IV | 1:400 | Ty. I<br>Ty. II<br>Ty. III | 1:25<br>1:50<br>1:25 | 18 hr. | 45° C. |
| Serum Agglutinating, Meningococcus Polyvalent | 1:400 | Types I, II, III, IV Gorden | | 18 hr. | 45° C. |
| Serum Agglutinating, Pneumococcus Type I | 1:8 | | | 30 min. | 37.5° C. |
| Serum Agglutinating, Pneumococcus Type II | 1:8 | | | 30 min. | 37.5° C. |
| Serum Agglutinating, Pneumococcus Type III | 1:8 | | | 30 min. | 37.5° C. |
| Serum Agglutinating, M. melitensis | 1:800 | | | 2 hr. | 37.5° C. |
| Serum Agglutinating, B. pestis | 1:800 | | | 2 hr. | 37.5° C. |
| Serum Agglutinating, B. pertussis | 1:800 | | | 2 hr. | 37.5° C. |
| Serum Agglutinating, B. plague | 1:800 | | | 2 hr. | 37.5° C. |
| Serum Agglutinating, B. typhosus | 1:6400 | Para A<br>Para B | 1:100<br>1:100 | 2 hr. | 37.5° C. |
| Serum Agglutinating, B. paratyphosus A | 1:6400 | B. typhosus<br>Para B | 1:100<br>1:100 | 2 hr. | 37.5° C. |
| Serum Aggluating, B. paratyphosus B | 1:6400 | Para A<br>Para B | 1:100<br>1:100 | 2 hr. | 37.5° C. |

2. *By using a Standard Serum.**

*Potency*

Freshly prepared serums must show as many agglutinin units as the Standard Serum when tested with any antigen.

*Specificity*

Each serum must show the same relative specificity as the Standard Serum when tested against antigens prepared from closely related types known to give group reactions.

## III. Standardization of Human Blood Typing Serum

*Type II Serum Standard*

Serum must agglutinate the corpuscles of a known type III individual and the clumping must be well under way in five minutes and complete in twenty minutes. The agglutination must be negative with a Type II individual.

*Type III Serum*

Serum must agglutinate the corpuscles of a known Type II individual and the clumping must be well under way in five minutes and complete in twenty minutes. The serum must also be entirely negative with Type III corpuscles after twenty minutes.

## IV. Hemolysins

*Antisheep Serum*

Serum should give complete hemolysis with a unit of 1 c.c. of 1:10,000, after one hour's incubation in the waterbath at 37° C. with 1 c.c. of a 1:20 dilution of the pooled

*Prepared as described under antigens.

serum of four or more guinea pigs, and 1 c.c. of a 2.5% suspension washed sheep cells. The sheep cell suspension is made up from defibrinated sheep blood washed four times with salt solution.

(It has been suggested by one member of the committee that 1:10,000 is too high for the minimum standard of potency, and that 1:5,000 is sufficiently high. Opinions are requested from members of the society.)

*Antisheep Serum dried on paper*

Two mm. x 5 mm. strip should give hemolysis after one hour's incubation in the waterbath at 37.5° C. with 1 c.c. of a 1:20 dilution of the pooled serum of four or more guinea pigs and 1 c.c. of a 2.5 per cent suspension of washed sheep cells. The sheep cell suspension is made up from defibrinated sheep blood washed four times with salt solution.

*Antihuman Serum*

Serum should give complete hemolysis with a unit of 0.1 c.c. of 1:100 after one hour's incubation in the waterbath at 37° C. with 0.4 c.c. of a 1:10 dilution of pooled guinea pig serum and 0.1 c.c. of a 5 per cent suspension of washed human red cells.

*Antihuman Paper*

Two mm. x 5 mm. strip should give hemolysis with 0.4 c.c. of a 1:10 dilution of pooled guinea pig serum and 0.1 c.c. of a 5 per cent suspension of washed human red cells.

V. Standardization of Pneumococcus Serums for the Precipitin Test

*Potency*

Undiluted serums must give, with a good precipitinogen, a well marked ring precipitate in 30 minutes in water-bath and a flocculent precipitate after shaking and further incubation for 30 minutes.

*Specificity*

Serums must be specific for their respective types after one hour's incubation (straight serum).

*Standard Antigen*

Twenty-hour bouillon culture in Hygienic Laboratory bouillon dissolved by addition of bile or sodium choleate solution.

VI. Standardization of Bacterial Antigens for Complement Fixation

Including:

Antigen, B. abortus Bang
Antigen, B. abortus Equine
Antigen, B. mallei
Antigen, Gonococcus
Antigen, B. pertussis
Antigen, B. tuberculosis

Method I*—By means of a Dried Standard Antigen prepared as follows:

1. Wash off growth in 50 per cent alcohol.
2. Centrifuge.
3. Add 50 per cent alcohol—stand 30 minutes 37° C.
4. Centrifuge.
5. Add ether, stir or shake, stand 1 hr. room temperature.
6. If ether yellow, wash again with ether.
7. Allow to dry. Should be perfectly dry.
8. White powder, store in small tubes tightly corked.

To use, suspend powder in salt solution in proportion.

One gram to 200 c.c. saline.

Freshly prepared antigens should not exceed in anticomplementary action, and should equal in antigenic action, the Dried Standard Antigen.

*Method of preparing standard Gonococcus Antigen. (Torrey) This method is acknowledged to be good for Gonococcus Antigen. Sufficient data could not be obtained for establishing a standard method for preparing other bacterial antigens for complement fixation. It is suggested by the chairman that the Society distribute experiments on this question to be worked upon and reports submitted at the next meeting.

Method II—By means of a Dried Standard Serum.

A serum of known potency is absorbed on filter paper and allowed to dry. It is cut into strips which are standardized with a good antigen and the fixation unit determined. The paper is stored in vacuum in a dark cool place.

A freshly prepared antigen is titrated for the anticomplementary unit. One third of this amount must give fixation with the serum paper unit.

## VII. Standardization of Antigens for the Wassermann Reaction

(Studies in the Standardization of the Wassermann Reaction, XXIX. Methods for Establishing a Uniform and Standardized Unit of Antigen. By John A. Kolmer, M.D., Philadelphia, Pa. The American Journal of Syphilis, Vol. VI, No. 4, October, 1922. Pages 664 and 665.)

The Use of Acetone Insoluble Lipoids Kept in Acetone or Dried Cholesterolized and Lecithinized Alcoholic Extracts for Establishing and Maintaining a Unit of Measure for the Titration of Antigen.

1. When the acetone insoluble lipoids are used, the mass is removed from the acetone and briefly fanned; 0.3 gm. is carefully weighed out on a piece of tinfoil and the whole placed in a small cylinder fitted with a ground-glass stopper. Two c.c. of highest grade ether and added and the residue dissolved; 9 c.c. of best grade acetone-free methyl alcohol are now added and well shaken. The extract is used unfiltered.

2. If dried antigen is to be used 5 c.c. amounts are placed in small vials with ground-glass stoppers and fanned to dryness. These are kept at room or refrigerator temperature. When used 5 c.c. of absolute ethyl alcohol are carefully added to a vial and the residue dissolved; the extract is used unfiltered.*

3. The antigen is now titrated for antigenic, anticomplementary and hemolytic activity; the smallest amount giving a ++++ reaction is called the antigenic unit. Kolmer has found this unit to keep unchanged for at least six months with both acetone insoluble lipoids and the dried.

4. When a new antigen is to be titrated and adjusted to this standard antigen, the titrations are conducted as follows:

(a) A fresh syphilitic serum (preferably a mixture of several sera) is heated at 55° C. for fifteen minutes and used in amounts of 0.02, 0.015, 0.01, 0.009, 0.008, 0.007, 0.006, 0.005, 0.004, 0.003, 0.002 and 0.001 c.c. with ten units of the standard or preserved antigen to determine the fixing unit or smallest amount of serum giving a ++++ reaction.

(b) The new antigen is titrated for hemolytic and anticomplementary activity.

(c) The standard antigen and new antigen are now titrated for antigenic activity using ten fixing units of the syphilitic serum. Each antigen is used in amounts of 0.5 c.c. of eighteen dilutions, from 1:100 to 1:1800. The smallest amount of each antigen giving a ++++ reaction is the antigenic unit. Of course the unit of standard antigen is likely to vary from its former unit because of a difference in the serum, but adjustment is made according to the following example:

Standard Antigenic unit 0.5 c.c. of 1:600 (0.00083 c.c.)
Unit of standard antigen in new titration 0.5 c.c. of 1:300 (0.0015 c.c.)
Unit of new antigen 0.5 c.c. of 1:900 (0.00055 c.c.)

$$\frac{0.00083 \text{ c.c.}}{0.0015 \text{ c.c.}} \times \frac{0.00055 \text{ c.c.}}{1} = 0.0003 \text{ c.c. or } 0.5 \text{ of } 1{:}1666$$

Dilution of the new antigen is equal to the standard unit and would be the new standard unit; ten units or 0.5 c.c. of 1:166 would be used in conducting complement-fixation tests for syphilis.

*This refers to use of a cholesterolized and lecithinized alcoholic extract of heart muscle. If dried acetone insoluble lipoids are used 0.3 gm. dissolved in 5 c.c. of ether should be placed in each vial and fanned to dryness. When used the residue is dissolved by adding 2 c.c. of ether followed by 9 c.c. of acetone-free methyl alcohol; the extract is used unfiltered.

(*Practical Physiological Chemistry, P. B. Hawk, 7th Ed. Blakiston, p. 638.)

Second example:
Standard antigenic unit 0.5 c.c. of 1:600 (0.00083 c.c.)
Unit of standard antigen in new titration 0.5 c.c. of 1:800 (0.000625 c.c.)
Unit of new antigen 0.5 c.c. of 1:700 (0.000715 c.c.)

$$\frac{0.00083}{0.000625} \times \frac{0.000715 \text{ c.c.}}{1} = 0.00095 \text{ c.c. or } 0.5 \text{ c.c. of } 1{:}526$$

Dilution of the new antigen is equal to the standard unit of 0.5 c.c. of 1:600 and could be used for the new standard antigen if the older antigen was exhausted or spoiled. In complement-fixation tests the new antigen would be used in dose of ten units or 0.5 c.c. of 1:53 if this amount were at least six times or more under the anticomplementary and hemolytic units.

5. In this manner new standard antigens can be adjusted as the original and preceding antigens are exhausted, the unit being constantly adjusted to the original.

### VIII. Standardization of Antigen for the Kahn Test

1. By means of a color standard.

For uniformity, Kahn recommends standardizing the alcoholic extracts to a given color range. This color range need not be absolute, but relative. A standard antigen for comparison may be obtained from Dr. Kahn or the following artificial color standards may be employed:

Standard 1:

1. 0.5 gm. Potassium Bichromate ($K_2Cr_2O_7$) is dissolved in 100 c.c. distilled water (permanent standard solution to be kept protected from light).
2. 1 c.c. of above solution is mixed with 75 c.c. of distilled water.
3. 10 c.c. of solution No. 2 is placed in a large test tube of about ¾ inch diameter. Into a tube of similar size is measured 10 c.c. alcoholic extract to be compared.

Standard 2:

1. An ammonium sulphate solution is prepared, 1 c.c. of which containing 0.01 mg. N.
2. 1 c.c. of this solution is measured into a large test tube about ¾ inch diameter, 8 c.c. of distilled water and 1 c.c. of Nessler's* solution added and mixed thoroughly.
3. Place 10 c.c. of alcoholic extract to be tested in a tube of similar size and compare colors.

2. By means of positive and negative sera.

Tests carried out with positive and negative sera should prove to be approximately not less sensitive than the Wassermann test. Furthermore, sera from untreated cases of secondary syphilis should give spontaneous reactions.

### IX. Standardization of Colloidal Gold Solution for the Lange Test

By means of known normal and known paretic spinal fluid.

The solution must not only have the proper color but must remain unchanged when run with a known normal spinal fluid, and give a typical curve with a paretic fluid.

Five c.c. of the solution must be completely precipitated in one hour by 1.7 c.c. of a 1 per cent solution of NaCl.

Five c.c. must be neutral to a 1 per cent solution of alizarin red in 50 per cent alcohol.

### X. Standardization of Reagents for Bass-Watkins Test

By means of standard serums.

10,000 million typhoid bacilli per c.c., killed and preserved with 1 per cent commercial formalin. Sealed and stored at 2-6° C. Must give definite agglutination within

*Practical Physiological Chemistry, P. B. Hawk, 7th Ed. Blakiston, P. 638.

two minutes with standard typhoid agglutinating serum in a dilution of 1-5. Must give negative reaction with standard Para A agglutinating serum and standard Para B agglutinating serum. Also must give negative reaction with serum which is negative to other standard methods.

By means of a standard antigen.

Freshly prepared antigens must react with the same rapidity and specificity as the standard antigen on any serum.

The committee was requested to continue its work and report at the next meeting.

The Society adopted a motion defining the term "Clinical Pathologist" as "a graduate in medicine who has specialized for three years or more in diagnosis of disease by laboratory methods including theoretical and a technical knowledge of clinical microscopy, medical chemistry, bacteriology, serology and tissue examinations."

A discussion of blood grouping led to the adoption by the society of the Moss classification as the official one to be recommended to the members. A referendum of those present showed that all employed the Moss system.

The rest of the morning session was devoted to a general discussion of the status of the clinical pathologist from a scientific as well as economic standpoint. The subject of an official publication was likewise taken up and a committee appointed to look into the matter.

## June 25, 1923—Second Session

DR. PHILIP HILLKOWITZ of Denver, Colorado, read his presidential address entitled **The Relation of the Clinical Pathologist to the Hospital.** (For original article see page 361.)

### DISCUSSION

*Dr. R. A. Kilduffe, Los Angeles, Cal.*—The standardization program has made a very acute and marked change in the laboratory situation in all hospitals. It has not been so long ago since the pathologist was in many instances merely a physician who wished to become a member of the staff, and who found that that was the only position not occupied, and who accepted it until there was an opening on the clinical side. However, these days are passing, and it is now realized in almost every hospital that the laboratory is practically the heart of the hospital, because, after all, without a correct diagnosis good work cannot be done. The one thing which it seems to me has been overlooked by hospitals, especially by the staffs of smaller hospitals has been that the director of the laboratory, in many instances, has been chosen mainly because of his ability to perform a great number of technical procedures. The emphasis has been laid upon the number of reactions which he was personally competent to do, rather than upon his ability to correlate the laboratory with the clinical side of the hospital. I feel that a competent director of laboratories in a hospital should not be looked upon, merely in the light of a technical performer, but that he is looked upon in that light is evidenced by the fact that even today one can find in the A. M. A. Journal advertisements which will state that a "technician" is wanted in the laboratory who must be a physician, a graduate of so and so, and so on. The director of a laboratory must not be looked upon primarily as a technician. Of course, he must have technical ability and experience or he will not be able to thoroughly and competently supervise the technical work which is going to be done, but it seems to me that his real function, and what he should aim to do, should be to act as a lliaison officer between the wards and the laboratory. I think the ideal situation, and it can be achieved no matter how small the hospital, would be, instead of presenting a requisition to the laboratory which covers everything from acne down to zymotic disease, when a dubious case comes into the hospital to have the clinician consult with the laboratory man and say, "Here is a dubious case; this is the history; here is the patient, now what in your opinion is the proper laboratory procedure to be set in motion which will be informative?" It is of little avail and of little value to simply accumulate on the chart a great mass of laboratory

reports, possibly only one or two of which are of the slightest interest or value. In that way, not only the time of the laboratory is conserved, but, if a fee rate is charged, the finances of the patient are looked after; real, good, informative work is done; and the patient is served by the united abilities of two men whose knowledge, while distinct in many particulars, must be correlated in many others. It seems to me that a good clinical pathologist must have a good clinical background. He must not only know how to make a certain test, but he must have a very clear and distinct conception of what the meaning of the test is after it has been made in order to interpret it in terms of John Smith or Bill Jones, or, in other words, the patient on whom the test is made.

One of the things Dr. Hillkowitz has mentioned, and in which the pathologist is no small factor, is the keeping of records. One thing, which I think is a very important consideration with regard to records is the fact that it is not enough to have very excellent records. They must be used. In other words, I think that not enough emphasis has been placed upon the function of the pathologist and the hospital records, from the standpoint of using the records and using the laboratory work as investigative material. I think sometimes it does not appear to be emphasized that while the function of the hospital primarily is to take care of the patients, it is secondarily, and of equal importance, its duty to provide a place in which new methods can be tried and possibly evolved; and, of course, as soon as we begin to think of investigation, we are led right into the laboratory without a moment's hesitation. I think the pathologist should not only discuss how the records shall be kept, and emphasize what laboratory investigation has been neglected perhaps, or what methods might be tried, but I think it is the function of the pathologist to say to the staff that they should invesigate this, that or the other idea. It may turn out not to be an idea, but at least that much will have been found out; and, of course, it should be up to the pathologist to secure from the staff clinical cooperation for these investigations.

Now, it is not necessary in utilizing the ordinary hospital for laboratory investigation that that hospital have a thousand beds, because twenty cases thoroughly sudied are much more informative and of much greater value than one thousand cases just glanced at, and the average hospital is more than willing to cooperate along these lines. Of course, the character of the laboratory staff, to repeat what Dr. Hillkowitz has said, is determined somewhat by the financial end of it. A man who is competent to run a laboratory as it should be run, who is competent not only to make tests, but to interpret them, to act as a good consultant—and that should be his status—is certainly worth a great deal more than the average hospital sees fit to pay. That fact is a matter for the pathologist to fight out with the hospial. I do not think we can standardize these things. I think if an individual is willing to work in a hospital for an amount of money which is absolutely inadequate, the fault rests not so much with the hospital that offers him the inadequate compensation, but with the individual who is satisfied to accept it and to remain in such a position. The important thing, it seems to me, and the unfortunate thing, is that no matter what the compensation may be to start with, it has a tendency to remain at that level regardless of what the pahologist may do for the hospital, regardless of the character of work he does or the reputation that he may manage to give that hospital by virtue of laboratory reports which are made or investigative work which he produces. Of course, all hospitals are always poor, they are always running on a deficit and there never seems to be much money for anybody. Therefore, in the matter of compensation it is absolutely impossible to get away from the fact that over and above a salaried compensation there must be an additional income derived from some place. Now, there are only two places from which it can come, one, the patient in the hospital, and in the second place, the outside patient not in the hospital, but for whom the pathologist will make a laboratory examination. Whether or not he is to get all of those fees or a percentage is something which he must also thresh out with the hospital itself. The important thing it seems to me is this, that I think the pathologist should absolutely rebel at being considered simply a person to whom anybody can come and have something done. I have always in my little way endeavored to feel and endeavored to make it felt that an individual sent to me for a serological examination, for example, was not coming to me to have the test made; he was coming to me exactly as he would go to an x-ray man or a nose and throat consultant,

or some other kind of consultant, to whom the general practitioner has referred him, and I have always endeavored to feel that I was not being paid for making a Wassermann, but for making it as correctly as I knew how to make it, and primarily for determining what that investigation stood for after it was made. But these, as I say, are all questions which the individual must confront as an individual. It is impossible to standardize them; it is impossible to solve them by resolution, or to produce any standard result. The two important things I feel in this question which should be standardized are, first of all, the fact that the director of laboratories, the pathologist in the hospital, should not be looked upon as a technician, and should not be chosen primarily for his manipulative skill, and secondly, if he does not utilize the material which is at his command for investigative work of the type which is feasible, if he does not do that, I do not feel that he does his full duty to the hospital, to the patient, or to himself, because if we do not endeavor to find out new information, we are bound to lose contact with the progress of pathology. If we do not learn something all the time, we very soon know very little, and I see no reason at all why even a hospital of moderate size such as I have generally been associated with, cannot conduct investigations of more or less interest. I have never had the slightest difficulty in interesting the medical and surgical sides of the staff in these problems, or in obtaining cooperation.

HARRY GAUSS, M.D., DENVER, COLO., **The Evolution of Clinical Pathology.** (Published in Journal of Laboratory and Clinical Medicine, August, 1923.)

WILLIAM CARPENTER MCCARTY, ROCHESTER, MINN., **The Pathologist of the Twentieth Century.** (For original article, see p. 365.)

L. H. CORNWALL, M.D., NEW YORK CITY, **Diagnostic Methods in Neurosyphilis.** (For original article, see p. 369.)

A. M. SAUNDERS, M.D., CHICAGO, ILL., **The Kottman Reaction on 500 Cases of the Insane.** (For original article, see p. 401.)

DISCUSSION

*S. A. Levinsohn, M.D., Chicago, Ill.*—The Kottman reaction seems to offer an index of thyroid activity, and we have employed it in clinical work. The test is simple, is rapidly carried out, and in over 400 serums examined, which were obtained from patients with thyroid dysfunction, as well as those ill with other diseases, it was observed that the Kottman reaction yielded a close index of the thyroid activity.

We have also conducted some experimental work with the Kottman reaction in which the thyroid gland was massaged, or subjected to roentgen-ray therapy, as well as the surgical removal of the thyroid gland. It was observed that after thyroidectomy, the Kottman reaction may return to normal. Experimentally, it was found that thyroxin injections, irradiation, and massage of the hypertrophied gland resulted in an increased protective property of the serum, which became evident in the course of several days. Bacterial or peptone shock, hemorrhage, or irradiation of remote organs, may alter the Kottman reaction, but this alteration is not comparable to that observed in the serums obtained from patients with exophthalmic goiter.

HERMAN L. SHARLITT, M.D., and WILLIAM G. LYLE, M.D., NEW YORK CITY, **The Measure of Urinary Sugar Following the Ingestion of a Standard Mixed Carbohydrate Breakfast.** (For original article, see p. 390.)

WILLIAM G. EXTON, NEW YORK CITY, **A Simple and Rapid Method for the Determination of Albumin in Urine with Preliminary Presentation of the Skopometer.** (Article follows.)

I feel that this method of testing albumin in the urine is so simple that perhaps but few words are necessary before an audience like this. Such a method gives considerable insight on the effects of therapy, diet and other features connected with the life of nephritics. This, in conjunction with the fact of checking the work of ten thousand people who are tested for albumin throughout the country, and finding the uncertain way in which it is done, and also, the accumulation of the mass of statistical material of insurance companies on the purely qualitative basis, have led us to try to find a method that would, as far as possible, be without the effect of a personal equation. We began this study by using a very unusual method, and on account of the great wealth of new albumin precipitants in connection with blood chemistry, we went through a good many. I shall not bother you with many of them; but may say we used two controls in working with all of these tests. In employing these controls we simply took a specimen that had a lot of albumin in it, and diluted it down progressively and recovered half the amount in each specimen. We found in working with the heat test that none of them were at all reliable when it came to getting anything like real quantitative results. Another control we used was to put a definite weight of albumin into a normal urine and try to recover the same amount of albumin in each sample.

As a result of all these experiments, we came to the conclusion that a good reagent is sulphosalicylic acid, but we found it was necessary to load this with a salt to give it a certain buffer action. The heat test is probably the best qualitative test. After we had worked out a reagent that could be used quantitatively, the question was how we were to measure these clouds, and we tried a great many things. We were not successful in developing a standard that was reasonably useful, but a representative of the Eastman Kodak Company succeeded in making a series of gelatine standards. These are about a year old, and they have held up very well. At that time, we started to investigate individuals who were continually passing a low-gravity urine, and yet did not show any other signs of nephritis. We wanted a sensitive method, and tried to use a nephelometer and other methods for calculating these clouds, but found we could not get consistent results with the nephelometer. This permitted us to measure one in fifty thousand. Beyond that, it was not sensitive, and it would have been necessary to have an instrument of an impracticable length. We were led to continue our experiments and to devise an instrument with which we were able to measure the difference between tap water and distilled water. This instrument is rather simple in theory, but it has given a good deal of trouble and there is a lot about it we don't know as yet, but we do know that we can take a specimen of urine, add equal parts of reagent to the urine, and in one minute, get an exact reading of the cloud, giving the quantity of the albumin. The principle of the instrument is practically that of the photometer. The lamp is adjustable to make up for differences in the lamps as they are manufactured. There are three little slides which hold targets. The target we use is a silverized piece of glass with a slit one-tenth of a millimeter wide when the lamp is lit—this is a special lamp with a six-inch filament which illuminates the target from below, and also gives a continual beam effect of scattered light in the square cup which we use in this instrument. The target is standard; the light is standard; the millimeter scale is the same, so that all you have to do is to put the cup in and when you look into the instrument, you see a little black field with a little hair line. The brightness is scattered by the particles until that matches the light which is allowed in from below. The field, of course, is perfectly light on account of the light reflected from the different particles.

This will afford us a means of making a complete quantitative test in about two minutes with an error not greater than 2 per cent.

*A Voice.*—Who makes the machine?

*Dr. Exton* (Continuing).—I really owe you an apology for showing you this one. It was made by mechanics in our laboratory. I have not made arrangements yet to farm it out to any particular concern, but I will do so soon. I will have that in my paper when it is ready.

DISCUSSION

*Dr. A. H. Sanford, Rochester, Minn.*—I want to say that I have followed with interest Dr. Exton's work in perfecting his precipitant for albumin. I have not followed all the different modifications of the skopometer; but I do know that the sulphosalicylic acid reagent is excellent. It is economical to use, and I have been convinced for some time that this is one of the best reagents to use in the clinical laboratory. Formerly, we used 20 per cent sulphosalicylic acid. This was difficult to obtain, and expensive. The new reagent, with increased specific gravity, and a small amount of sulphosalicylic acid, is far more economical and simple to use.

*John A. Kolmer, M.D., Philadelphia.*—I have been very much interested in Dr. Exton's reagent in Philadelphia. I would like to ask Dr. Exton whether he uses the reagent for examination of the spinal fluid. While I am not prepared to say just what will react with the normal amount of protein in the spinal fluid, I know it to be used for detecting the increase of protein.

*Dr. William G. Exton, New York City.*—We haven't very much spinal material to examine, but I am very glad to hear you say what you did, because two men in New York who have tried it have come to the same conclusion that you have. The subject of albumin in urine is rather difficult to cover in ten minutes, and there are many features connected with the working out of the test, and determining what a real albumin test should be. Almost any protein-like substance will cause a cloud similarly with phosphoric acid and a lot of other things. We also have been able to get very rapid estimations of the amount of albumin in the blood. I think with this method, we will have a way of separating albumin fractions, globulin fractions, and getting it quantitatively within a short time. We have used it for salt or chloride determinations quite regularly, and we are working on a method of determining urea.

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Official Organ of the American Society of Clinical Pathologists



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## EDITORIALS

### *Endocrine Control of the Kidney*

DR. W. LANGDON BROWN has enunciated an unusually interesting theory of the control of renal function exerted by certain of the endocrine organs. It is based primarily upon recent studies in comparative anatomy and embryology, and although the experimental evidence is necessarily limited, the theory is of such general interest as to warrant discussion.

The primitive excretory organs among invertebrates are the nephridia, distributed segmentally and opening directly into the body cavity or upon the external surface. Among the worms, they are alike in each segment but in the arthropods they become differentiated according to location. Among the crustaceans the anterior group of nephridia becomes concentrated into the "green gland," which still retains an excretory function, secreting into the alimentary tract. It will be recalled that according to Gaskell's theory of the development of vertebrates from arthropods, the alimentary tract becomes the central canal of the nervous system. Those nephridia or their derivatives in the higher invertebrates which had emptied into this original alimentary tract now find themselves without an external outlet. The anterior group becomes the pituitary. The second group, in the neighborhood of

the gill-slits, eventually develop into tonsils, thyroid, parathyroid and thymus. In the process of development the primitive nephridia can apparently take on either lymphoid or glandular structure. According to Gaskell the uterus in limulus has a similar position and arrangement to that of the thyroid with its thyroglossal duct in ammocetes. "The relationship which has been known from time immemorial to exist between the sexual organs and the thyroid in man and other animals and has hitherto been a mystery without any explanation, may possibly be the last reminiscence of a time when the thyroid glands were the uterine glands of the paleostracan ancestor."

While the pituitary body originated from the closure of the original alimentary canal, those glands derived from the second group of nephridia have become ductless because of the altered ectodermal topography associated with the formation of the new alimentary tract. It is suggested that the pronephric and mesonephric tubules of the embryo again represent the segmental distribution of the original nephridia.

The cortical portion of the adrenal gland is said to be derived from the mesonephridial tubules, while the medulla takes its source from the sympathetic ganglia. "This striking union of two structures of such widely different origin as the cortex and medulla of the adrenals must have an important advantage because once it was achieved in evolution it was never dropped. While if it fails to occur in fetal life, the brain remains in the fish stage, the neopallium does not develop, and an anencephalic monster results. I would suggest that the success of an adrenal graft into the testis in Hurst's case of Addison's disease might have been due to its insertion into a tissue of similar origin, the adrenal cortex and the interstitial cells both arising from the Wolffian body, which is of nephridial origin."

The close developmental relationship between the gonads and the renal anlagen is well known.

Brown suggests that the influence of the endocrine glands on the kidney is in part at least an expression of the interest they continue to take in functions which were formerly their own.

"Maccallum maintains that the earliest function of the kidney was not so much the elimination of waste products as the regulation of the inorganic composition of the blood. While nephridia opened into the body cavity the external medium of the sea and the internal medium naturally tended to be closely similar. But with the shutting off of the nephridia from the body cavity by the intrusion of a vascular tuft the animal was set free from the tyranny of its external medium, could individualize its metabolism, as it were, and change its habitat without altering the conditions under which its tissues and organs worked. This independence of external media is far more characteristic of the vertebrates than of the invertebrates, and the change from nephridia to kidneys has thus been of special importance in the evolution of vertebrates. This independence is maintained even when a mammal returns to marine life, so that whereas the oceanic forms of invertebrate life tend to have the same saline concentration as the sea, the whale retains very much the blood salinity of the pig. The development of a cir-

culation which enabled excretion to be adequate even when confined within a small compass was an important factor in rendering this change possible. When nephridia were replaced by a more adaptable mechanism and some of their outlets were dammed up by the formation of new structures such as the vertebrate alimentary tract and the pleural folds, they either had to vanish altogether or to take on some new function. To a large extent the second course was adopted and some of them became endocrine glands."

Just as the original function of the kidneys was probably regulatory rather than excretory and dealt with the inorganic composition of the blood, the endocrine organs still exert primarily a regulatory function, a specialization in each gland of those functions which were formerly distributed equally among the primitive nephridia. In general the internal secretions serve to maintain the equilibrium of the various metabolic processes of the body and to maintain a balance with regard to the external environment. The active principle of the thyroid secretion is an iodine compound of indol which in turn is a decomposition product of tryptophane, a constituent of the protein molecule.

"Indol is ordinarily split off by the intestinal bacteria, so that it looks as if we were dependent on these parasites, particularly Bacillus coli, for the first step in the preparation of an important hormone; one, indeed, which is essential to adult development. During the nursling period the infant gets its thyroid secretion from its mother's milk, and during that time the Bacillus coli is hardly ever present in its bowel. The suckling child has not a very active pancreatic secretion, and therefore cannot split off tryptophane very easily from protein and thus does not form indol to any extent.

"This would fit in very well with Herter's views as to a form of infantilism due to persistence of the intestinal flora of the nursling period. It would appear that the body cannot only detoxicate indol by converting some of it into indican, but can actually change it into a defensive substance. This would explain McCarrison's observations on the influence of intestinal intoxication on thyroid enlargement. It is reminiscent of the primitive excretory functions of the thyroid to find indol conjugated with iodine in its secretion, while indol conjugated with potassium sulphate continues to be excreted as indican by the kidney.

"It is also interesting to note that tyramine, which is produced from the putrefaction of tyrosin in the human alimentary canal, is closely similar in chemical structure and action to adrenalin. It tempts one to refer the formation of adrenalin to the tyrosin of food proteins, just as thyroxin arises from tryptophane, another aromatic group in food protein. But it should be noted that the body is capable of making its own tyrosin, and that in alkaptonuria, a congenital error of metabolism, although the body is unable to utilize the tyrosin of the food, symptoms of hypoadrenalism do not occur. Nevertheless, I anticipate that further research may show that other endocrine secretions are modifications of excretory products, just as endocrine glands are modified excretory structures. Other hints are to be found in such things as the diuretic effect of thyroid extract by dehydration of the

body fat; the destruction of a purin body such as guanidine and the regulation of the calcium ions in the blood by the parathyroids. To sum up this part of my argument, Maccallum's theory may be restated, that the principal function of the primitive nephridia was to bring the internal working of the organism into harmony with its environment. Now surely this is exactly what the endocrine system continues to do, both through its secretory activity and through its close association with the emotional, i.e., the sympathetic nervous system. In this way a further division of labor is achieved, the kidney being set free from the labor of forming an internal secretion, and able to devote itself entirely to excretion. But at the same time we should expect to find that the other internal secretions would be able to influence the work of the kidney in order to insure harmony between the internal economy and its environment. This is particularly well seen in the persistent relationship between the pituitary and the kidney. This is of biological interest because the pituitary represents a gland which was definitely excretory in function in invertebrates as high as the crustacea, and is of clinical interest as bearing on diabetes insipidus and polyuria in general."

The remainder of Brown's paper deals chiefly with the action of pituitrin upon the secretory activity of the kidneys. Its action in diminishing the polyuria of true diabetes insipidus is well known. He concludes that from the evidence in hand it appears highly probable that pituitrin acts upon the renal cells in such a way as to regulate the threshold of the kidney for water.

Materials excreted by the kidneys fall into two categories: purely waste products, such as ammonia, urea and uric acid, for which there is no threshold of excretion; and those which may play a useful part in cellular life, such as sugar, sodium chloride, hemoglobin and water, against which the kidney interposes a barrier or threshold. The threshold is not constant for any one substance, and it seems possible that its height may be controlled by certain internal secretions. Water is of value to cellular life and its renal threshold is apparently controlled by the pituitary secretion. In hyperthyroidism we observe rise in the threshold for sugar. It has been suggested that the calcium drainage in the ketosis of diabetes is due to failure of the parathyroids which cooperate in some respects with the pancreas. It is not certain that this is due, however, to any alteration of the renal threshold for calcium.

"Claude Bernard urged that the object of all vital mechanisms was to keep the internal environment constant. We have seen that nephridia originally were the principal means of maintaining a constant relationship between the internal and external environments. In evolution nephridia were replaced by analogous, but probably not homologous, tubules which form the kidney, the function of which may be defined as that of keeping the chemical composition of the blood constant. Some nephridia were retained and modified to house the hormones, which, previously less specialized and more widely distributed, now play an important part in maintaining the internal environment."

Many features of this attractive theory are not subject to proof at the present time, but it is interesting to speculate thereon, and it may well be that further studies of comparative anatomy, embryology and physiology will corroborate the theory at least in part.

REFERENCE

Brown, W. Langdon: Proc. Royal Society of Medicine, (Sec. of Urology) Dec., 1923, xvii, 1.

—*W. T. V.*

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## *The American Society of Clinical Pathologists*

The formation of this society was stimulated by:

1. The necessity of picking out the practical diagnostic and therapeutic laboratory facts and methods from those of pure scientific interest;

2. The necessity of standardization of these facts and methods;

3. The necessity of setting a standard educational requirement for laboratory consultants to obviate the rapid increase of false claims of technicians who frequently conduct commercial laboratories, the value of which many physicians are unable to determine;

4. The necessity of maintaining laboratory sciences within the realm of the profession of medicine and not allowing them to become merely commercial adjuncts;

5. The stimulation of the interest of the general profession in the real value of the laboratory in the practice of medicine and surgery;

6. To assist in the standardization of hospitals.

In the three years of the Society's existence it has already brought together many laboratory consultants who were rapidly drifting away from contact with the active practitioners of medicine; it has prepared programs of practical discussions which have not only drawn laboratory experts but general practitioners to their meetings. This active cooperation of interest and mutual benefits is the chief object of this Society.

The members of the organization appreciate the recent affiliation of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE as its official organ for its transactions.

—*Wm. Carpenter MacCarty, M.D.*

# BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

## *Manual of Chemistry*

THE twelfth edition of this work like previous editions, offers a general review of physical chemistry, general chemistry, analytical chemistry—both qualitative and quantitative—and organic chemistry. The inclusion of all of these subdivisions of chemistry within 642 pages necessitates some abridgment and the work would therefore be considered rather elementary for those devoting their entire time to a study of chemistry. For medical students and others who must acquire a fairly general knowledge of chemical principles in the various branches, the book should have a genuine value, and should be of particular use to those who desire a single reference book on chemistry as a whole. The reviewer feels that greater space might advantageously be devoted to the phenomena of chemical physics or physical chemistry.

## *Circulation in Health and Disease*†

THE second edition of Dr. Wiggers' monograph has been thoroughly revised and brought up to date. This has necessitated a very appreciable increase in the size of the volume. Dr. Wiggers' viewpoint is always that of the research student working in the laboratory and as a consequence, his work contains more pure science than do many other current works upon the heart, in which the clinical aspects tend to dominate the consideration. Perhaps a natural consequence is that the book does not make as easy reading as do some of the others.

Although case reports and similar clinical considerations receive minor consideration it must be said that the book is not without distinct value to the clinician who can acquire herefrom a clearer understanding of the cardiovascular mechanism. Section one deals with the physiology of the circulation, section two with graphic methods for the clinician, and section three with diseases of the heart and circulation, in which are included detailed discussions relative to hemoglobin, shock, plethora, cardiac strain,

*Manual of Chemistry. By W. Simon, Ph.D., M.D., Late Professor of Chemistry in the College of Physicians and Surgeons of Baltimore and in the Baltimore College of Dental Surgery; and Daniel Base, Ph.D., Formerly Professor of Chemistry in the Maryland College of Pharmacy, Department of the University of Maryland, Baltimore. Twelfth edition, thoroughly revised. With fifty-five illustrations, one colored spectra plate and six colored plates representing forty-eight chemical reactions. Pp. 642. Cloth. Price $5.00. Lea and Febiger, Phila., and New York, 1923.

†Circulation in Health and Disease. By Carl J. Wiggers, M.D., Professor of Physiology in the School of Medicine of Western Reserve University, Cleveland, Ohio. Cloth. Price, $7.50. Pp. 662. Second edition. 204 engravings. Lea & Febiger, Phila. and New York, 1923.

cardiac reserve, oligemia, arteriosclerosis, hypertension, hypotension, etc.

Cardiologists could scarcely consider their libraries complete without this volume.

The bibliography is very complete.

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## *Practical Chemical Analysis of Blood**

THIS book, originally the outgrowth of a series of articles appearing in the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, has been enthusiastically received and has, as a consequence, rapidly passed into a new and enlarged edition. In the first edition the author limited himself chiefly to a presentation of those methods which he has found most serviceable in the quantitative estimation of the various constituents of the blood. In the second, the general plan is the same, but the discussion of other methods is of broader scope and the Folin-Wu system of blood analysis is described in detail. Recently developed methods for the quantitative determination of inorganic constituents of the blood are incorporated. In fact it may be said that the chemical examination of the blood is considered from every available angle. This includes, among the other studies in more common use, estimations of acid-base balance, hemoglobin, oxygen content and oxygen capacity, and acetone bodies.

The significance of results is discussed in connection with each subject, and a separate chapter deals with the blood chemical findings in certain diseases. A valuable chapter deals with quantitative methods of urinalysis. The appendix contains formulae for standard reagents, logarithm tables, etc. The second edition is a welcome contribution.

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## *Medical and Veterinary Entomology†*

THIS volume is intended primarily as a textbook for the use of medical and veterinary students. It should find a distinctly wider field of usefulness. The subject matter is treated with unusual thoroughness and at the same time in a very readable manner. There are few of us who are not naturally interested in bugs in general, and unfortunately many are perforce interested in such as infest our living quarters, clothing or persons. The first four chapters are devoted to an historical review, discussion of parasitism as an abstract phenomenon, the classification of insects, and general anatomic considerations. Chapter five discusses, perhaps rather too briefly, how insects cause and carry disease. The remainder and greater bulk of the book is devoted to descriptions of the individual groups of insects such

*Practical Chemical Analysis of Blood. By Victor C. Myers, Ph.D., M.A., Professor and Director of the Department of Biochemistry, New York Post-Graduate School and Hospital. Cloth. Price $5.00. Second revised edition. Illustrated. Pp. 232. C. V. Mosby Company, St. Louis, 1924.

†Medical and Veterinary Entomology. By William B. Herms, Professor of Parasitology in the University of California; Consulting entomologist for the California State Board of Health, and formerly professor of Zoology and Parasitology in the San Francisco Veterinary College; Member National Malaria Committee. Author of "Malaria, Cause and Control," "A Laboratory Guide to the Study of Parasitology," "The House fly in its relation to the Public Health," "House fly Management," etc. Second Edition. Completely revised. Cloth. Price $3.50. Pp. 462. The Macmillan Company, New York, 1923.

as those represented by cockroaches and beetles, lice, bed bugs, mosquitoes, buffalo gnats and horse flies, the house fly, control, fleas, ticks, mites and venomous insects. The anatomy, habits, life history, distribution, relation to disease transmission and control of each species is discussed. The illustrations, which are abundant, are chiefly photographic reproductions and are unusually well done.

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### *Laboratory Diagnosis of Syphilis**

THIS volume serves particularly as a vehicle for the exposition of the work of the author and his associates, in the laboratory diagnosis of syphilis. This probably was not the primary reason for its publication, but the work would seem to have been more complete had space been given to discussion of certain other laboratory procedures such as Kolmer's quantitative reaction, and the flocculation or precipitation tests for syphilis. Aside from this, the work should become a valuable reference book for serologists.

The phenomena of hemolysis and of antigen and antibody formation are first considered, with their application to the diagnosis of syphilis. The author discusses the standard Wassermann reaction and that developed in his laboratory. He mentions the use of homohemolytic systems. In a chapter on the diagnostic value of the serum reaction, the author tabulates the statistical conclusions of various workers. He further discusses the effect of treatment upon the reaction. Additional chapters are devoted to the luetin reaction, examination of the cerebrospinal fluid and methods for the detection and study of Treponema pallidum. The use of stains in the recognition of treponema is described at some length. There are some excellent photomicrographs.

The index is rather brief. The typography is excellent.

*Laboratory Diagnosis of Syphilis. By Hideyo Noguchi, M.D., M.S., Ph.D., D.Sc., Member of the Rockefeller Institute for Medical Research, New York. Cloth. Price $7.50. Pp. 392. Fifty-nine illustrations. New York, Paul B. Hoeber, Inc., 1923.

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## *NEWS AND NOTES*

### The Third Annual Meeting of the American Society of Clinical Pathologists

The members of the American Society of Clinical Pathologists are looking forward with pleasurable anticipation to the next annual meeting which will be held in Rochester, Minn., June 5, 6 and 7, 1924. The time chosen is the latter part of the week prior to the annual convention of the A. M. A. which is to be held in Chicago, thus making it possible for clinical pathologists to attend both meetings without inconvenience or loss of time.

What is in store at the next A. S. C. P. meeting under the hospitable auspices of the Mayo Clinic may be glimpsed from the following letter sent out to the members by President Wm. C. MacCarty:

"Dear Doctor:

"We are arranging the program for the meeting of the American Society of Clinical Pathologists which will occur June 5-7, 1924, in Rochester, Minnesota.

"The mornings will be devoted to practical demonstrations of diagnostic laboratory methods (laboratories of hematology, parasitology, metabolism, physiology, cardiography, serology, physiological chemistry, biophysics, radiology, roentgenology, bacteriology, clinical microscopy, surgical pathology, morbid anatomy, cancer cytology, dietetics, diabetes, and general medical chemistry).

"The afternoons will be devoted to papers by members of the Society. There will naturally be a limited number of papers, but we want, if possible, to HAVE EVERY MEMBER OF THE SOCIETY LET US KNOW WHAT HE IS ESPECIALLY WORKING ON THAT HE MAY PRESENT HIS OBSERVATIONS IN THE FORM OF A PAPER.

"Will you please let us know if you can have something to present? We are not certain that all papers can be presented at the meeting, but your desire to read a paper and your special interest will greatly assist us in arranging program and discussions.

"This meeting will be characterized by a clear demonstration to the profession of the actual value of the laboratory to the practice of medicine.

Yours fraternally,

(Signed) William Carpenter MacCarty, President."

A cordial invitation to participate in the convention is also extended to nonmembers—clinical pathologists and physicians who are interested in any phase of laboratory diagnosis.

Numerous inquiries have reached the secretary of the A. S. C. P. from prospective members who desire information as to the requirements and eligibility to membership in this organization.

The following excerpts from the Constitution and By-Laws may be useful to those who desire affiliation with the A. S. C. P.

Article II—Objects

The objects of this society shall be: (a) To promote the practice of scientific medicine by a wider application of clinical laboratory methods to the diagnosis of disease; (b) to stimulate original research in all branches of clinical laboratory work; (c) to establish from time to time uniform standards for the performance of various laboratory examinations; (d) to elevate the scientific and professional status of those specializing in this branch of medicine; (e) to encourage a closer cooperation between the practitioner and the clinical pathologist.

Article III—Membership

Section 1. The membership of this society shall consist of (a) active, (b) associate, (c) honorary, and (d) corresponding members.

Section 2. Active members shall be graduates in medicine from a recognized medical school, who have specialized in clinical pathology for at least three years and who are devoting the major portion of their time to this field.

Section 3. Associate members shall be graduates of recognized scientific institutions, who have made valuable contributions to any of the sciences relating to clinical Pathology, or whose association with this organization will further the objects of this society. Associate members shall pay the regular dues and have the privilege of voting but not of holding office.

**Application blanks and other information relating to the organization may be had from Dr. Ward Burdick, Secretary, 652 Metropolitan Bldg, Denver, Colorado.**